From Department of Medicine, Huddinge
Karolinska Institutet, Stockholm, Sweden

HUMAN CYTOTOXIC LYMPHOCYTE DIFFERENTIATION IN HEALTH AND DISEASE

Heinrich Schlums

Karolinska Institutet

Stockholm 2017

Published by Karolinska Institutet.
Printed by AJ E-print AB, Stockholm

ISBN 978-91-7676-804-4

HUMAN CYTOTOXIC LYMPHOCYTE DIFFERENTIATION IN HEALTH AND DISEASE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

PUBLIC DEFENSE:

LECTURE HALL 9Q, ALFRED NOBELS ALLÉ 8

KAROLINSKA UNIVERSITETSSJUKHUSET HUDDINGE

FRIDAY NOVEMBER 17TH, 2017, 09.30AM

By

Heinrich Schlums

Principal Supervisor:
Assistant Professor Yenan T. Bryceson
Karolinska Institutet
Department of Medicine, Huddinge
Center for Hematology and Regenerative Medicine (HERM)

Co-supervisor:
Professor Hans-Gustaf Ljunggren
Karolinska Institutet
Department of Medicine, Huddinge
Center for Infectious Medicine (CIM)

Opponent:
Professor Adrian Hayday

Affiliation 1:
King's College London
Department of Immunobiology

Affiliation 2:
Francis Crick Institute, London
Immunosurveillance Laboratory

Examination Board:
Professor Cecilia Söderberg-Nauclér
Karolinska Institutet
Department of Medicine, Solna

Professor Susanna Cardell
Gothenburg University
Department of Microbiology and Immunology

Assistant Professor Jonathan Coquet
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

ABSTRACT

Cytotoxic lymphocytes, comprising natural killer (NK) cells and $CD8^+$ cytotoxic T cells, eradicate infected or malignant cells by release of lytic granules and alarm the immune system through production of pro-inflammatory cytokines and chemokines. NK cells and $CD8^+$ T cells belong to different arms of the immune system, employing complementary strategies for target cell recognition. As part of the innate arm, NK cells sense missing or induced self-molecules by an array of germline-encoded activating and inhibitory cell surface receptors. In contrast, adaptive $CD8^+$ T cells depend on somatically recombined, clonally distributed T cell receptors (TCR) that recognize unique foreign peptides presented by MHC class I on target cells. Importantly, while mature NK cells readily kill target cells without prior sensitization, naive $CD8^+$ T cells require antigen priming to differentiate into cytotoxic effector and long-lived memory cells, providing long-term protection against re-infection. Recently, memory features including longevity and recall responses have also been ascribed to differentiated NK cell subsets. The work presented in my thesis contributes to our molecular understanding of cytotoxic lymphocyte differentiation processes in health and disease.

Exocytosis of lytic granules containing cytotoxic cargo is tightly controlled, but the transcriptional regulation of the factors governing degranulation is poorly understood. In **paper I**, we found that expression of one of those factors, Munc13-4, was induced upon cytotoxic lymphocyte maturation and required cooperative binding of the transcription factors ELF1 and STAT4 to an evolutionary conserved region in intron 1. Transcription factor-binding facilitated chromatin remodeling and DNA accessibility, allowing for enhanced transcription of the conventional as well as induction of a newly identified, alternative Munc13-4 isoform that is likely to play a central role in lymphocyte cytotoxicity.

Infection with cytomegalovirus (CMV) in mice and man is associated with expansion and persistence of NK cell subsets with enhanced effector function. In **paper II**, we show that such adaptive NK cells display previously unappreciated phenotypic and functional heterogeneity, and provide a molecular definition of such diverse subsets. Human adaptive NK cells lacked expression of the intracellular signaling molecules FcεRγ, SYK and EAT-2 as well as the transcription factor PLZF, thereby altering the signaling properties of key NK cell surface receptors and the responsiveness to innate cytokines, respectively. Silencing of signaling protein expression correlated with promoter DNA methylation and global DNA methylation patterns of adaptive NK cells approximated those of differentiated $CD8^+$ cytotoxic effector T cells. Importantly, adaptive NK cells failed to kill activated, autologous T cells, implying a functional specialization towards immunosurveillance of infected cells. Moreover, utilizing samples from patients with bone marrow disorders associated with *GATA2* haploinsufficiency (**paper III**) or acquired *PIGA* mutations in hematopoietic stem cells (**paper IV**), we demonstrate that adaptive NK cells are long-lived and can persist in settings of hematopoietic stem and progenitor cell attrition where canonical NK cells are lost.

Tissue-resident memory T (T_{RM}) cells provide early, localized adaptive immunity in non-lymphoid tissues. In **paper V**, we discovered a functional dichotomy of $CD8^+$ skin T_{RM} cells based on expression of the marker CD49a. Upon stimulation, $CD49a^+$ cells produced IFN-γ and acquired cytotoxic potential by induction of the lytic granule constituents perforin and granzyme B. Primed $CD49a^+$ T cells accumulated in the dermis and epidermis of vitiligo lesions, an autoimmune condition characterized by local depigmentation as a result of melanocyte destruction. In contrast, $CD49a^-$ T cells produced IL-17 and were enriched in lesional skin from psoriasis patients, promoting local inflammation.

These insights shed light on novel mechanisms controlling human cytotoxic lymphocyte differentiation and may thus be of potential benefit to health.

LIST OF SCIENTIFIC PAPERS

I. Cichocki, F., **Schlums, H.**, Li, H., Stache, V., Holmes, T., Lenvik, T.R., Chiang, S.C.C., Miller, J.S., Meeths, M., Anderson, S.K., Bryceson, Y.T., 2014. **Transcriptional regulation of Munc13-4 expression in cytotoxic lymphocytes is disrupted by an intronic mutation associated with a primary immunodeficiency.** *J. Exp. Med. 211, 1079–1091.*

II. **Schlums, H.***, Cichocki, F.*, Tesi, B., Theorell, J., Beziat, V., Holmes, T.D., Han, H., Chiang, S.C.C., Foley, B., Mattsson, K., Larsson, S., Schaffer, M., Malmberg, K.-J., Ljunggren, H.-G., Miller, J.S., Bryceson, Y.T., 2015. **Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function.** *Immunity 42, 443–456.*

III. **Schlums, H.***, Jung, M.*, Han, H., Theorell, J., Bigley, V., Chiang, S.C.C., Allan, D.S.J., Davidson-Moncada, J.K., Dickinson, R.E., Holmes, T.D., Hsu, A.P., Townsley, D., Winkler, T., Wang, W., Aukrust, P., Nordøy, I., Calvo, K.R., Holland, S.M., Collin, M., Dunbar, C.E., Bryceson, Y.T., 2017. **Adaptive NK cells can persist in patients with GATA2 mutation depleted of stem and progenitor cells.** *Blood 129, 1927–1939.*

IV. Corat, M.A.F.*, **Schlums, H.***, Wu, C., Theorell, J., Espinoza, D.A., Sellers, S.E., Townsley, D.M., Young, N.S., Bryceson, Y.T., Dunbar, C.E., Winkler, T., 2017. **Acquired somatic mutations in PNH reveal long-term maintenance of adaptive NK cells independent of HSPCs.** *Blood 129, 1940–1946.*

V. Cheuk, S., **Schlums, H.**, Gallais Sérézal, I., Martini, E., Chiang, S.C., Marquardt, N., Gibbs, A., Detlofsson, E., Introini, A., Forkel, M., Höög, C., Tjernlund, A., Michaëlsson, J., Folkersen, L., Mjösberg, J., Blomqvist, L., Ehrström, M., Ståhle, M., Bryceson, Y.T., Eidsmo, L., 2017. **CD49a Expression Defines Tissue-Resident CD8(+) T Cells Poised for Cytotoxic Function in Human Skin.** *Immunity 46, 287–300.*

* authors contributed equally

CONTENTS

LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cellular cytotoxicity
AML	Acute myeloid leukemia
APC	Antigen-presenting cell
BLIMP-1	B-lymphocyte-induced maturation protein-1
BRG1	Brahma-related gene 1
BTB-ZF	Broad-complex, tramtrack and bric-à-brac - zinc finger
CD	Cluster of differentiation
CMV	Cytomegalovirus
CTL	Cytototoxic T lymphocyte
DAP12	DNAX activating protein of 12 kD
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DNAM-1	DNAX accessory molecule-1
DNMT	DNA-methyltransferase
EAT-2	EWS-Fli1-activated transcript-2
EBV	Epstein-Barr virus
ELF1	E74-like factor 1
EOMES	Eomesodermin homolog
ER	Endoplasmatic reticulum
FcεRγ	Fc-epsilon receptor 1 subunit gamma
FHL	Familial hemophagocytic lymphohistiocytosis
GATA-2	GATA-binding protein-2
GPI	Glycosylphosphatidylinositol
HAT	Histone acetyltransferase
HCT	Hematopoietic cell transplantation
HCV	Hepatitus C virus
HDAC	Histone deacetylase
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSPC	Hematopoietic stem and progenitor cell
HSV	Herpes simplex virus
IFN	Interferon
IL	Interleukin
ILC	Innate lymphoid cell
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
ITSM	Immunoreceptor tyrosine-based switch motif
KIR	Killer-cell immunoglobulin-like receptor

LFA-1	Lymphocyte function-associated antigen 1
MAIT	Mucosa-associated invariant T cell
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
NCR	Natural cytotoxicity receptor
NK	Natural killer
NKT	Natural killer T
PAMP	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cell
PID	Primary immunodeficiency
PIGA	Phosphatidylinositol N-acetylglucosaminyltransferase subunit A
PLZF	Promyelocytic leukemia zinc finger protein
PNH	Paroxysmal nocturnal hemoglobinuria
RNA	Ribonucleic acid
SAP	SLAM-associated protein
SLAMFR	Signaling lymphocytic activation molecule family of receptors
STAT	Signal transducer and activator of transcription
SYK	Spleen tyrosine kinase
T-bet	T-box protein expressed in T cells
TCR	T cell receptor
T_{CM}	Central memory T cell
T_{EM}	Effector memory T cell
T_N	Naïve T cell
T_{RM}	Tissue-resident T cell
TGF	Transforming growth factor
TNF	Tumor necrosis factor
VZV	Varicella zoster virus
ZAP-70	Zeta-chain-associated protein kinase 70

1 INTRODUCTION

The world is a dangerous place. The human body is constantly exposed to a myriad of threats from both the outside, in the form of infectious agents such as viruses and bacteria, as well as the inside in the form of malignant transformation, which may result in cancer. Cells of the immune system are specialized to sense changes in the healthy environment and protect against infection and malignancy throughout the body. A first line of defense provided by rapidly responding cells of the innate immune system recognizes conserved, pathogen associated molecular patterns (PAMPs) and danger signals in affected tissues. These innate immune cells display broad specificity but have defined roles in the early immune response and include granulocytes (neutrophils, basophils, eosinophils and mast cells), innate lymphoid cells such as natural killer (NK) cells, as well as macrophages and dendritic cells (DCs). Neutrophils engulf and destroy pathogens, while basophils, eosinophils and mast cells release inflammatory mediators such as cytokines, enzymes, histamine and reactive oxygen species. NK cells directly lyse infected or transformed cells. Macrophages and DCs phagocytose and digest pathogens or cellular debris for presentation to T cells of the second line of defense, the adaptive immune system. B and T lymphocytes of the adaptive immune system possess antigen-specificity mediated by clonally distributed, somatically rearranged antigen receptors. They expand massively upon activation and provide long-lasting protection against re-infection through formation of persistent memory cells. B cells bind soluble antigen and produce antibodies whereas $CD4^+$ and $CD8^+$ T cell subsets recognize peptides presented in the context of MHC molecules and orchestrate immune responses through cytokine production or killing of infected or neoplastic cells, respectively [1].

1.1 Cytotoxic Lymphocytes

Cytotoxic lymphocytes can kill target cells by directed release of cytotoxic granules containing perforin, a pore-forming homomultimeric protein, and granzymes, serine proteases initiating apoptosis pathways upon delivery to the cytoplasm of target cells [2]. The major cytotoxic lymphocyte subsets in humans are natural killer (NK) cells and $CD8^+$ T cells. While NK cells belong to the innate immune system and acquire cytotoxic potential during development, adaptive $CD8^+$ T cells differentiate into cytotoxic effectors upon engagement of MHC class I molecules with cognate antigen in the context of cytokine stimulation during infections.

In addition to target cell killing, cytotoxic lymphocytes can produce the pro-inflammatory cytokines interferon γ (IFN-γ) and tumor necrosis factor (TNF) as well as chemokines such as macrophage inflammatory protein-1β (MIP-1β) [3]. IFN-γ has anti-viral, anti-mycobacterial and growth inhibitory properties and promotes MHC class I/II expression as well as T-helper 1 differentiation [4]. TNF stimulates the release of other pro-inflammatory mediators, facilitates recruitment and maturation of myeloid cells, and has direct cytotoxic effects [5]. MIP-1β mainly functions to mobilize other innate and adaptive inflammatory cells to sites of injury or infection [6].

This thesis aims to expand our understanding of cytotoxic lymphocyte differentiation processes, how such processes contribute to protecting the host from infection and how imbalances in these processes may cause disease. This could help in developing tools to manipulate or harness cytotoxic lymphocyte responses in chronic infection, autoimmune disorders and cancer.

1.1.1 NK Cells

Today, NK cells are considered part of a larger group of innate lymphoid cells (ILCs) characterized by the lack of somatically recombined antigen receptors found on adaptive B and T cells. While NK cells mediate cytotoxicity, the other ILCs are grouped according to their cytokine-mediated roles in immune responses against pathogens and formation of secondary lymphoid tissues [7,8].

NK cells constitute 5-15% of peripheral blood mononuclear cells (PBMCs) and were first described in mice in 1975 by two independent laboratories at the Karolinska Institutet, Stockholm and the National Institutes of Health, Bethesda [9,10]. Kiessling *et al*. and Herberman *et al*. reported large granular lymphocytes isolated from spleen and other lymphoid organs with the ability to rapidly kill leukemia and tumor cells in culture without prior sensitization within one to four hours. Shortly after, first evidence for a central role of MHC class I in NK cell-mediated target cell recognition resulting in bone marrow and tumor graft rejection emerged [11]. Klas Kärre and colleagues finally proposed an alternative, more primitive immune defense strategy complementary to T cells, which was based on NK cell detection of MHC class I levels on aberrant or foreign cells [12]. This “missing-self” hypothesis stipulated that NK cell activation occurs upon absence or reduced expression of self-MHC class I, in contrast to cytotoxic T cells which rely on TCR-mediated recognition of peptides presented by MHC class I. Down-regulation of MHC class I poses a powerful immune evasion strategy employed by viruses and tumor cells to avoid T cell-mediated destruction. Therefore, detection of missing or reduced MHC class I by NK cells serves to discriminate between self and non-self and complements adaptive immunity.

Today, the concept of “missing-self” is well established and many of the receptors binding MHC class I have been discovered. In an example of convergent evolution, mouse NK cells express inhibitory Ly49 receptors for detection of MHC [13] while human NK cells sense MHC quality and quantity largely via the family of inhibitory killer cell immunoglobulin-like receptors (KIRs) [14]. A multitude of mostly uniformly expressed, germline-encoded activating receptors that bind stress-induced ligands or ligands indicating “self” complement the inhibitory receptors in target cell recognition. Thus, the lack of inhibitory or an increase in activating signals promotes NK cell activation. More recently, inhibitory receptors have been implicated in a process termed “education” or “licensing” [15,16], which tunes NK cell reactivity depending on the net signaling strength generated during sub-threshold interactions with healthy cells [17]. This ultimately results in a spectrum of activation thresholds determined by the combination of activating and inhibitory receptors on a given NK cell and the ligands expressed on the surface of the other host cells at steady-state [18–20].

Besides their function as innate killers in early defense against viruses and cancer, NK cells rapidly produce large amounts of IFN-γ, TNF and MIP-1β, thereby instructing and shaping the immune response (see above). NK cells also relay signals from other immune cells by producing IFN-γ in response to combinations of exogenous cytokines such as interleukin (IL)-12, IL-15, IL-18 and IFN-α [21]. Importantly, immunoregulatory killing of activated immune cells by NK cells is vital to maintain immune homeostasis as evidenced by primary immunodeficiencies (PIDs) such as familial hemophagocytic lymphohistiocytosis (FHL), caused by genetic defects in lymphocyte cytotoxicity [22,23]. Furthermore, the innate functionality of NK cells is harnessed to mediate graft-versus-leukemia (GvL) effects in settings of hematopoietic stem cell transplantation and cancer immunotherapy [24].

Since their discovery more than 40 years ago, many fundamental aspects of NK cell biology such as modes of activation and target cell recognition have been uncovered. Furthermore, an impressive body of work has been accumulated describing the origin and development of early NK cell progenitors as well as the differentiation into diverse, unique repertoires of mature NK cell subsets that can by found in circulation and various peripheral tissues. Importantly, the idea of NK cells as strictly innate effectors has been challenged. Recent findings ascribe features of immunological memory to specialized NK cell subsets after exposure to pathogens, in particular cytomegalovirus (CMV).

NK cell receptors and signaling

In contrast to T and B cells, which are largely governed by a single, somatically recombined antigen receptor, NK cell function is determined by the sum of signals provided by an array of germline-encoded activating and inhibitory surface receptors. While most activating receptors are uniformly expressed on mature NK cells, expression of inhibitory receptors appears stochastically distributed and changes upon terminal differentiation [25]. Importantly, with the exception of CD16, most activating NK cell receptors are “co-activating” or “co-stimulatory” in nature. Insufficient to trigger effector functions in freshly isolated, resting NK cells by themselves, co-engagement of at least pairwise combinations of receptors is required for cytokine production and degranluation [26]. The molecular basis for such receptor synergy has recently been uncovered and co-stimulation is needed to overcome a threshold for Vav1 activation set by the E3 ubiquitin ligase c-Cbl [27,28]. Furthermore, the signals for cytolytic granule exocytosis and polarization are uncoupled. Engagement of the β2 integrin LFA-1 by ICAM-1 is necessary for granule polarization and directed release to facilitate target cell lysis with minimal killing of bystander cells [29,30].

The majority of inhibitory NK cell receptors expressed at steady-state bind classical or non-classical MHC class I molecules found on all nucleated healthy human cells in a non-peptide specific manner [31]. As MHC class I alleles may or may not be expressed in a given individual and inhibitory receptor expression on NK cells is variegated, tolerance is ensured by “disarming” cells that do not possess receptors for self-MHC. In contrast, NK cells expressing self-binding inhibitory receptors become “educated” or “licensed” to produce cytokines and degranulate against target cells with reduced MHC class I surface levels as a result of infection, transformation or stress [18,19]. While the concept of NK cell education is well established, the underlying molecular and cellular mechanisms are still incompletely understood. Guia *et al.* proposed that tonic inhibitory signals promote the reorganization of activating receptors into membrane nanodomains necessary for full NK cell reactivity [32]. Moreover, very recent data suggest a role for higher basal activity of the mTOR/Akt pathway that correlates with the number of inhibitory receptors on educated murine NK cells [33].

The most prominent inhibitory receptors expressed by human NK cells are members of the large family of inhibitory killer cell immunoglobulin-like receptors (KIRs), NKG2A and LILRB1 (CD85j). Inhibitory KIRs bind alleles of the classical MHC class I molecules HLA-A, B or C [14] and are mostly expressed at later stages of mature NK cell differentiation as discussed further below. In contrast, the inhibitory receptor NKG2A, which localizes to the cell surface as a heterodimer with CD94, is mostly found on earlier stages of NK cell maturation and binds the non-classical MHC class I molecule HLA-E [34–36]. Stabilized by leader peptides of classical MHC class I molecules, HLA-E levels depend on peptide availability and NKG2A therefore senses MHC class I expression indirectly [37]. Inhibitory CD85j is mostly found on terminally differentiated NK cells and, except for its high affinity ligand HLA-G, binds to a broad variety of

classical and non-classical MHC class I molecules with lower affinity compared to inhibitory KIRs or NKG2A.

Inhibition by the receptors above is mediated by one or several immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails. Src-family kinases such as Lck phosphorylate the central tyrosine of an ITIM upon receptor engagement, leading to recruitment of phosphotyrosine phosphatases SHP-1 and SHP-2 via their tandem SH2-domains [38]. These negative regulators intercept early activating signals, probably by dephosphorylating Src- and SYK-family kinases, although this still remains to be shown in primary human NK cells. In fact, only the guanine nucleotide exchange factor Vav1, a regulator of the actin cytoskeleton, has been identified as SHP-1 substrate after NK cell co-culture with HLA-C expressing target cells [39]. Dephosphorylation of Vav1 after inhibitory receptor engagement likely blocks re-organization of the actin mesh, thereby obstructing formation of a stable immune synapse as well as recruitment and clustering of activating receptors [31,40–42]. Concomitantly, inhibitory receptors have also been shown to trigger phosphorylation of the signaling adaptor Crk by the tyrosine kinase c-Abl, resulting in dissociation of the signaling complex regulating actin re-organization [43].

A portion of activating NK cells receptors possess only small intracellular domains and couple to immunotyrosine-based activation motif (ITAM)-containing adaptor molecules for signaling. CD16 and the natural cytotoxicity receptors (NCRs) NKp30 and NKp46 can form complexes with homo- as well as heterodimers of FcεRγ or CD3ζ. In contrast, NKG2C and activating KIRs as well as the NCR NKp44, which is expressed on IL-2 activated peripheral blood and resting uterine NK cells, couple to DAP12 homodimers [44–46]. The low affinity Fc-receptor CD16 (FcγRIIIa) can be found on the majority of human peripheral blood NK cells and allows for the destruction of IgG opsonized target cells by antibody-dependent cellular cytotoxicity (ADCC) [47]. As CD16 function depends on adaptive B cell responses the receptor is not considered to confer natural cytotoxicity, which per definition relies entirely on innate receptor-ligand pairs. In contrast, the NCRs NKp30, NKp44 and NKp46 bind a variety of known and unknown cellular as well as viral and bacterial ligands on transformed or infected cells [48]. Importantly, the NKp30 agonist B7-H6 is expressed on many tumor cell lines and is induced on monocytes and neutrophils upon stimulation with TLR-ligands or pro-inflammatory cytokines [49,50]. NKp30 thus not only plays a role in immunosurveillance of infected and transformed cells but also contributes to immunoregulation of activated immune cells. Similar to its inhibitory counterpart, NKG2C is expressed as heterodimer with CD94 and binds the non-classical MHC class I molecule HLA-E [35,36]. NKG2C therefore has an important function in sensing virus infection via reduced MHC class I but stabilized or elevated HLA-E levels, an immune evasion strategy adopted by for instance HCMV, HCV and HIV [51–53]. Despite the similarities of inhibitory and activating KIRs as a result of gene duplication, only some activating KIRs have been shown to bind MHC class I molecules with low affinity and ligands for the majority of receptors remain unknown [46]. Thus, the precise role of activating KIRs in NK cell biology still needs to be uncovered. Similar to ITIMs, the central tyrosine-residues of ITAMs get phosphorylated by Src-family kinases upon receptor engagement, which results in recruitment of SYK-family kinases SYK or ZAP-70. These phosphorylate the scaffold proteins LAT and SLP-76 as well as other signaling molecules downstream, ultimately leading to influx of Ca^{2+} ions from the extracellular space and activation of central regulators of NK cell function. These include the MAP kinases ERK and p38 as well as the transcription factors NFκB and NFAT [54,55].

Among others, important non-ITAM-coupled activating receptors on resting NK cells include NKG2D (CD314), members of the SLAM-family of receptors (SLAMFRs) and DNAM-1 (CD226). NKG2D binds to stress ligands induced by infection, transformation or DNA damage

and plays a prominent role in cancer surveillance, as NKG2D-deficient mice are more likely to develop spontaneous tumors [56]. NKG2D-ligands include the MHC class I-like molecules MICA and MICB as well as members of the ULBP family [57]. NKG2D couples to the transmembrane adaptor protein DAP10 [58], which is able to recruit either the p85 subunit of PI3K or a Grb2-Vav1 complex for NK cell activation and cytotoxicity [59–62].

SLAMFRs are broadly expressed on hematopoietic cells and, with the exception of 2B4 (CD244) which binds CD48, form homophilic interactions *in trans* [63]. Resting NK cells express the SLAMFRs CD84, Ly9 (CD229), 2B4 (CD244), CRACC (CD319), and NTB-A (CD352). SLAMFRs possess immunoreceptor tyrosine-based switch motifs (ITSMs) within their cytoplasmic tail and can bind positive or negative regulators of cell signaling. The small SAP-adaptors SAP and EAT-2 propagate activating signals by recruiting the Src-family kinase Fyn or the phospholipases PLCγ1 and PLCγ2, respectively [63–66]. Additionally, SAP-adaptors prevent binding of negative regulators of cell signaling such as SHP-1, SHP-2, SHIP and Csk to the ITSM [67]. Importantly, genetic defects in *SH2D1A* encoding SAP underlie X-linked lymphoproliferative disease type I (XLP1). Characterized by defects in T and NK function and hyperinflammation, XLP1 underscores the importance of regulating activated immune cells to prevent pathology [68,69]. Recently, the inhibitory potential of SLAMFRs has also been shown to contribute to NK cell education in mice and XLP1 patients [70,71].

Similar to NKG2D, the receptor DNAM-1 prevents spontaneous tumor formation and tumor growth in mice [72] and has a critical role in NK recognition and killing of freshly isolated human cancer cells [73–75]. DNAM-1 binds the nectin adhesion molecule CD112 and the poliovirus receptor CD155, which are upregulated on tumors but also activated dendritic and T cells [76–78]. Interestingly, DNAM-1 physically and functionally associates with the β2 integrin LFA-1 and DNAM-1 surface levels on a given NK cell positively correlate with the quality and quantity of educating signals provided by inhibitory receptors for self [79,80]. The cytoplasmic domain of DNAM-1 was recently shown to recruit the adaptor Grb2 via an ITT-like motif. This was sufficient to activate of Vav1, PI3K and PLCγ1 as well as promote activation of Erk, Akt and calcium influx downstream [81].

Curiously, NK cells express seemingly redundant pairs of signaling molecules and adaptors of which individual members are usually associated with specific hematopoietic lineages. While FcεRγ, SYK and PLCγ2 are typically expressed in B- or myeloid cells, CD3ζ, ZAP-70 and high levels of PLCγ1 are restricted to T cells. Despite some evidence for PLCγ isoform preference downstream of specific NK cell receptors [82], results obtained from PLCγ2 deficient mice and humans with PLCγ2 mutations suggest a central role for PLCγ2 in NK cell function [83,84]. In contrast, SYK or ZAP-70 alone are sufficient for signaling downstream of ITAMs but show differences in dependency on upstream Src-family kinases [85]. A somewhat unique pair, the SAP-family adaptors SAP and EAT-2 have distinct roles in recruiting different signaling molecules to SLAMFRs. In humans and mice, SAP is found in T cells while EAT-2 appears to be NK cell specific although low expression can be detected in murine macrophages and dendritic cells [86]. This NK cell specific profile of SAP family adaptors suggests a unique role for SLAMFRs in NK cell biology and function, in particular regarding surveillance of hematopoietic cells [87]. Overall, the reason for pairwise expression of signaling proteins in NK cells compared to other lineages remains to be elucidated. We hypothesized that differential expression of signaling molecules upon NK cell differentiation may provide an additional layer of regulation and means of functional diversification (**paper II**).

NK Cell Development

As lymphocytes, NK cells develop from $CD34^+$ hematopoietic stem cells (HSCs) and bone marrow as a source of NK cell progenitors was first postulated shortly after their discovery [88]. To determine a more specific NK cell progenitor, mature human NK cells were later generated from $CD34^+HLA\text{-}DR^-$ and $CD34^+CD7^+$ cells isolated from bone marrow [89,90], from $CD34^+CD3^-CD4^-CD8^-$ fetal thymocytes with NK and T cell potential [91] as well as from $CD34^+CD38^+$ fetal liver cells without T cell potential [92]. Using additional surface markers such as CD56, CD16, CD94, CD7 and NKR-P1A (CD161) Jaleco *et al*. proposed a model for NK cell development based on their findings in fetal liver. This was later expanded and refined by Freud and colleagues in the laboratory of Michael Caligiuri investigating human bone marrow, lymph nodes and tonsils [93–95]. The model by Freud *et al*. describes five discrete stages of human NK cell development from $CD34^+CD45RA^+$ bone marrow precursors in secondary lymphoid tissues. There, progenitors undergo progressive NK cell commitment as a result of cues provided by the local environment. IL-15 in particular has been shown to be instrumental for NK cell development [96] and mutations in the IL-15 receptor affecting the common γ-chain CD132, the IL-2R/IL-15Rβ chain CD122 or defects in the signaling molecule JAK3 downstream of the IL-15 receptor manifest in severe combined immunodeficiency with loss of NK cells [97–100]. While the developmental stages 1-3 contain early progenitors and immature NK cells, stage 4 and stage 5 are considered mature, functional NK cells.

With the discovery of other, non-cytotoxic ILC subsets the search for a committed NK cell precursor without ILC potential shifted into focus as considerable overlap between NK cell and ILC developmental stages and sites exist [101]. Recently, an early NK cell-lineage restricted progenitor without ILC potential was identified in fetal and adult tissues [102]. Furthermore, Freud *et al*. provided an updated view of NK/ILC development by introducing the developmental stages 4a and 4b, defined by expression of the surface receptor NKp80 [103]. Stage 4b $NKp80^+$ were cytotoxic and expressed higher levels of T-bet and EOMES. In mice, T-bet and Eomes control central checkpoints of NK cell maturation [104] and cooperatively induce high expression of CD122. This allows for IL-2/15 responsiveness important for development of effector functions and survival [104,105]. In contrast, stage 4a $NKp80^-$ cells produced IL-22 and expressed higher levels of ILC3-associated transcription factors [103].

NK Cell Differentiation and Function

Mature stage 4 and 5 NK cells in peripheral blood and secondary lymphoid organs can be identified by differential surface expression of the neural cell adhesion molecule (NCAM) CD56 and the low affinity Fcγ-receptor CD16 while uniformly lacking T cell-associated CD3 [106,107]. Simplified, stage 4 cells are $CD56^{bright}CD16^-$, while stage 5 is characterized by a $CD56^{dim}$ and mostly $CD16^+$ phenotype. The existence of intermediate phenotypes led to the proposal that $CD56^{dim}$ derive from $CD56^{bright}$ NK cells. Indeed, different laboratories provided evidence for shorter telomere length in $CD56^{bright}$ compared to $CD56^{dim}$ cells as well as the potential of $CD56^{bright}CD16^-$ cells to upregulate perforin and CD16 and to differentiate into $CD56^{dim}CD16^+$ NK cells upon *in vitro* activation or transfer into NOD-SCID mice [108–111]. Furthermore, $CD56^{bright}$ NK cells are among the first lymphocytes to repopulate after hematopoietic stem cell or umbilical cord blood transplantation and acquire CD16 expression and increased cytolytic function over time [112–114].

Approximately 10% of peripheral blood NK cells are $CD56^{bright}$, while this ratio is reversed in lymph nodes and tonsils. $CD56^{bright}$ cells uniformly express the secondary lymphoid tissue homing markers CCR7 and CD62L, whereas $CD56^{dim}$ NK cells lack CCR7 and show

variegated CD62L expression [21,115,116]. Functionally, $CD56^{bright}$ NK cells are considered mostly immunoregulatory, producing larger amounts of IFN-γ and other soluble factors than $CD56^{dim}$ cells upon stimulation with combinations of monocyte-derived cytokines such as IL-12, IL-15, IL-18 and IL-1β [116]. They express lower levels of the cytotoxic molecules perforin and granzymes and less efficiently form conjugates with susceptible targets cells [117]. Furthermore, as they lack expression of inhibitory KIRs but uniformly express NKG2A, $CD56^{bright}$ NK cells are less well equipped to perform immunosurveillance of virus-infected cells. In line with this notion, the lack of CD16 also renders them incapable of antibody-dependent cellular cytotoxicity (ADCC) of infected cells.

While $CD56^{bright}$ NK cells constitute a relatively homogeneous population, peripheral blood $CD56^{dim}$ NK cells display much more diverse phenotypes as a result of continued differentiation leading to differences and specialization regarding function, target cell recognition and activation thresholds. The expression of CD62L on $CD56^{dim}$ NK cells has been associated with a functionally intermediate stage characterized by abundant IFN-γ production and proliferative capacity after cytokine stimulation as well as strong natural and antibody-dependent cytotoxicity and cytokine production after stimulation with susceptible target cells [118]. Furthermore, three independent labs described the terminal differentiation of $CD56^{dim}$ NK cells based on loss of NKG2A but acquisition of KIR and CD57 expression [114,119,120]. Differentiation along this axis is accompanied by gradual loss of proliferative capacity and IFN-γ production after IL-12+IL-18 stimulation while expression of cytotoxic granule constituent as well as target cell killing, degranulation and cytokine production after CD16 engagement increase. Terminal differentiation of $CD56^{dim}$ NK cells thus seems to direct NK cell function towards immunosurveillance of infected cells. Importantly, differentiating cells also acquire expression of the receptor NKG2C and the ability to respond to HLA-E expressing target cells [114]. Recently, “memory-like” features of $NKG2C^{+}$ NK cells as an adaptation to CMV infection have increasingly become the focus of interest and will be discussed in more detail in the next section and **papers II-IV**.

Further highlighting the heterogeneity of mature NK cells, phenotypically and functionally distinct tissue-resident NK cells can be found in the liver and female reproductive tract, where they aid in pathogen surveillance or regulate placentation, respectively [121–123].

Adaptive NK cells

Herpesviruses such as herpes simplex viruses (HSV) type 1 and 2, Epstein Barr Virus (EBV) or Cytomegalovirus (CMV) are ubiquitous in nature and establish life-long latent infections with opportunistic reactivation in large proportions of people in all parts of the world. While infections in the healthy are often asymptomatic and go unnoticed, newborns and immunocompromised individuals unable to control the viruses may develop severe and potentially lethal pathology. Herpesviruses have co-evolved with their hosts for millions of years and have developed mechanisms to avoid detection and clearance by the immune system [124]. Cytomegalovirus in particular devotes a vast number of genes to manipulate and evade the immune response [125]. In return, the human innate as well as adaptive immune system have developed countermeasures to keep these infections in check. NK cells appear at the center of controlling herpesvirus infections [126] and primary immunodeficiencies (PIDs) marked by NK cell loss or dysfunction result in susceptibility to herpesviruses in man [127,128]. Surprisingly, in the absence of T cells, NK cells alone can be sufficient to confer protection from lethal CMV infection as evidenced by a patient with mutations in the IL-7 receptor α chain resulting in a $T^{-}B^{+}NK^{+}$ SCID [129]. Notably, the vast majority of NK cells detected in this case study were $NKG2C^{+}$.

In mice (C57BL/6), NK cells expressing the activating, DAP12-couple receptor Ly49H expand up to 1000-fold and persist after a contraction phase for several months following MCMV infection [130–134]. Theses cells display increased IFN-γ production and degranulation after re-challenge and confer protection from MCMV upon adoptive transfer into naïve mice [134]. Ly49H directly binds the virus-encoded protein m157 [135,136] and m157-deficient mutants fail to induce expansion and memory-like responses [134].

Evidence for a similar role of $NKG2C^+$ NK cells in detection of HCMV infection first arose after phenotyping PBMCs from healthy CMV^+ or CMV^- donors and aviremic HIV^+ individuals [137,138]. In these studies, Guma *et al*. could show that seropositivity for CMV but not other members of the herpesvirus family correlated with temporally stable increases in frequencies of $NKG2C^+$ NK cells. Of note, Guma *et al*. also reported decreased surface expression of CD161 as well as the NCRs NKp30 and NKp46, while frequencies of inhibitory KIRs and $CD85j^+$ cells were elevated in CMV^+ donors. Shortly after, the same group demonstrated that fibroblasts actively infected with CMV could trigger expansion of $NKG2C^+$ NK cells *in vitro* when co-cultured in the presence of IL-15 [139]. This effect was inhibited by addition of anti-CD94 antibodies during co-culture, supporting the notion of a direct involvement of the CD94/NKG2C dimer in sensing acute CMV infection. This was followed by findings in patients undergoing solid organ or hematopoietic stem cell transplantation [140,141]. CMV reactivation in these patients led to stable expansions of differentiated, $CD57^+NKG2C^+$ NK cells with increased potential to produce IFN-γ compared to $NKG2C^-$ cells, implicating a functional adaptation after pathogen exposure *in vivo*. Indeed, evidence for epigenetic changes by demethylation of the *IFNG* locus was later shown to be at least partially responsible for the increase in cytokine production in $NKG2C^+$ NK cells after CMV infection [142]. Of note, expansions of $NKG2C^+$ NK cells have also been described in individuals with acute Hantavirus [143] and chronic Hepatitis B or C virus [144] infections, but only occurred in CMV^+ patients, underlining the central role of CMV for their development.

While murine “memory-like” NK cell properties have mostly been ascribed to the $Ly49H^+$ compartment [134], human “adaptive” NK cells appear to be more diverse. NK cells co-expressing DAP12-coupled activating KIRs and inhibitory self-KIRs besides NKG2C were shown to expand and persist in CMV^+ donors [145]. At roughly the same time, the group of Sungjin Kim reported elevated frequencies of NK cells lacking expression of the intracellular signaling adaptor FcεRγ in CMV^+ individuals [146,147]. These cells displayed greatly increased cytokine responses when stimulated with anti-CD16 antibodies or when co-cultured with HCMV or HSV-1 infected target cells in the presence of serum containing virus-specific antibodies [146–148]. In contrast, $Fc\varepsilon R\gamma^-$ NK cells responded poorly to tumor targets, indicating a special role in recognition of virus-infected cells. $Fc\varepsilon R\gamma^-$ NK cells also expressed elevated levels of anti-apoptotic Bcl-2 and cell frequencies were stable over time for up to nine months, supporting the notion of memory-like behavior of these cells. Importantly, $Fc\varepsilon R\gamma^-$ NK cells uniformly showed decreased expression of NKp30 and NKp46 but were not entirely contained within the $NKG2C^+$ population, emphasizing the potential diversity of human adaptive NK cells (**Figure 1**).

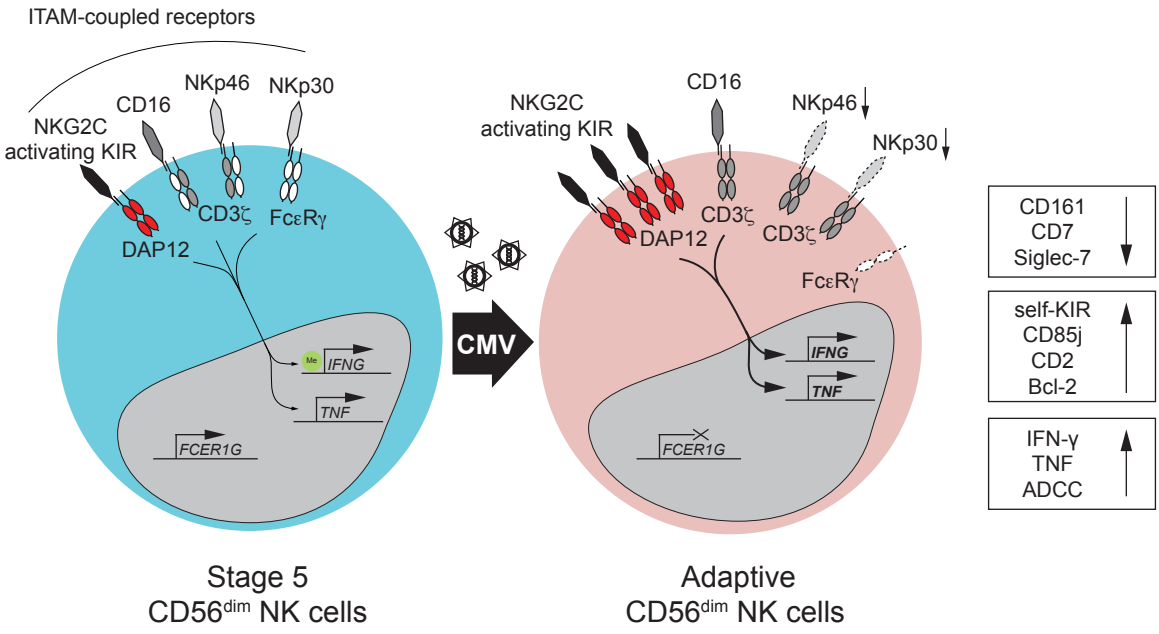

Figure 1. Phenotypic and functional characteristics of human adaptive NK cells. CMV-associated adaptive NK cells often express DAP12-coupled NKG2C or activating KIRs and inhibitory receptors for self-MHC Class I (self-KIR). Compared to canonical "stage 5" CD56^{dim} NK cells, adaptive NK cells uniformly express high levels of CD85j and CD2 while downregulating CD161, CD7, Siglec-7, NKp30 and NKp46 surface expression as well as expression of the intracellular signaling adapter FcεRγ. Functionally, adaptive NK cells display increased cytokine production upon CD16 stimulation compared to canonical CD56^{dim} NK cells, which can at least partially be explained by hypomethylation of the *IFNG* locus. Elevated expression of Bcl-2 may facilitate long-term survival similar to memory T cells.

The origin and the precise instructive signals for the development and survival of human adaptive NK cells remain largely unknown. In *Nfil3*-deficient mice lacking conventional NK cells, MCMV infection drives the development and expansion of Ly49H$^+$ NK cells with memory features in an m157-dependent manner similar to wildtype (WT) mice [149]. Blocking IL-12 and/or type I IFN signaling with monoclonal antibodies prevented formation of the memory pool, indicating a critical role for pro-inflammatory cytokines in this process. Similarly to WT cells, memory-like NK cells were dependent on IL-15 signaling for homeostasis and survival [149]. In a follow-up study, Beaulieu *et al*. found that the transcription factor Zbtb32 was highly upregulated early during viral infection [150]. Responsible for the proliferative burst but not survival of Ly49H$^+$ NK cells, expression of Zbtb32 was induced by IL-12, IL-18 and type I IFN signaling leading to STAT4 activation and binding to the *Zbtb32* promoter. Mechanistically, Zbtb32 antagonized the anti-proliferative transcription factor Blimp-1 allowing for the expansion of virus-specific NK cells. In line with these observations, Rölle *et al*. could demonstrate a similar role for monocyte-derived IL-12 in the expansion of human NKG2C$^+$ NK cells when co-cultured with CMV infected fibroblasts. Blockade of either IL-12 or CD94/NKG2C signaling significantly inhibited the proliferation of NKG2C$^+$ cells [151]. In mice, engagement of DNAM-1 during MCMV infection also poses a requirement for the differentiation of memory-like NK cells [152] but a role for DNAM-1 in human adaptive NK cell differentiation remains to be demonstrated.

Although the phenotypic and functional heterogeneity of human NK cells were well appreciated, deeper insights into the potential diversity of adaptive NK cell responsiveness as well as surface receptor and signaling molecule expression were lacking. We thus set out to investigate the phenotypic, functional as well as molecular changes that accompany NK cell

differentiation in response to CMV infection (**paper II**). Furthermore, we addressed the aspect of adaptive NK cell longevity by utilizing peripheral blood samples form patients with heterozygous mutations in *GATA2* (**paper III**) as well as individuals with paroxysmal nocturnal hemoglobinuria (PNH) (**paper IV**).

GATA2 encodes the transcription factor GATA-binding protein-2 (GATA-2) required for survival, homeostasis and proliferation of hematopoietic stem and progenitor cells (HSPCs) [153,154]. Importantly, GATA-2 haploinsufficiency as a result of heterozygous loss-of-function mutations in *GATA2* can manifest in hypocellular bone marrow failure and immunodeficiency with progressive loss of monocytes, DCs, B and NK cells [155,156]. Patients may thus develop severe mycobacterial, papilloma and herpes virus infections [157,158], but also present with myelodysplastic syndrome (MDS) that can evolve to acute myeloid leukemia (AML) [159–162]. Curiously, while *GATA2* deficiency is associated with loss of immature $CD56^{bright}$ NK cells, some patients accumulate terminally differentiated $CD56^{dim}$ NK cells [163,164]. We thus speculated that NK cells in these patients represent long-lived adaptive NK cells that persist after attrition of the stem cell pool. To this end, we performed extensive phenotypic and functional analysis of NK cells in *GATA2* patients and asymptomatic carriers (**paper III**).

Employing a similar strategy to investigate adaptive NK cell homeostasis and survival, we examined PBMCs from patients with PNH caused by acquired loss-of-function mutations of *PIGA* in HSPCs [165]. As *PIGA* encodes phosphatidylinositol *N*-acetylglucosaminyltransferase subunit A, a critical component of the synthesis pathway of glycosylphosphatidylinositol (GPI) anchors, patients produce mature hematopoietic cells without GPI-anchored membrane proteins. GPI^+ and GPI^- HSPCs may co-exist in the bone marrow and produce progeny for years giving rise to mixed phenotypes of peripheral blood cell populations. For unknown reasons, GPI^- HSPCs may progressively and completely dominate replenishment of lineages with shorter half-lives such as neutrophils and red blood cells. T cell lineages largely maintain GPI^+, likely a result of homeostatic self-renewal of mature cells in the periphery. We hypothesized that potentially long-lived adaptive NK cells would retain GPI-anchors while canonical NK cells with an estimated half-life of 14 days [166] originate from GPI^- HSPCs. Therefore, we used adaptive NK cell markers established in **paper II** in combination with stainings for GPI-anchors to determined the distribution of canonical and adaptive NK cell populations within GPI^+ and GPI^- subsets in PNH patients (**paper IV**).

1.1.2 T cells

Together with antibody-producing B cells, T cells belong to the adaptive arm of the vertebrate immune system. They are characterized by antigen specificity and the ability to form memory cells, which mount rapid responses upon pathogen re-challenge. Antigen specificity and T cell activation are dictated by somatically recombined, clonally distributed T cell receptors (TCR) that recognize foreign peptides presented by MHC molecules on the surface of antigen presenting or target cells. The TCR is a heterodimer of either αβ- or γδ-chains encoded by *TCRA* and *TCRB* or *TCRG* and *TCRD* loci, respectively. The TCR propagates intracellular signals via ITAM-containing CD3 adaptors, in particular CD3ζ, within the TCR-complex. Constituting approximately 95% of total T cells, the two most abundant subsets use αβTCRs and can be distinguished by surface expression of the co-receptors CD4 and CD8 [167,168].

$CD4^+$ T helper (T_H) cells recognize peptides in the context of MHC class II on professional antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages, and differentiate into potent cytokine producers to instruct and orchestrate the immune response

[169]. $CD8^+$ T cells sense foreign peptides presented by MHC class I molecules, which are expressed on all healthy cells of the body except for red blood cells. Upon activation in secondary lymphoid organs, mature but antigen-inexperienced ("naïve") $CD8^+$ T cells proliferate and differentiate into effector cells with the potential to kill infected or neoplastic cells and produce IFN-γ and TNF. The stages of $CD8^+$ T cell development and differentiation into cytotoxic effector and memory cells will be discussed in more detail below.

T cell development

T cell precursors develop from common lymphoid progenitors in the bone marrow and migrate to the thymus where they mature into different T cell lineages. Crucial checkpoints during these processes involve the recombination of TCR genes and selection of T cell clones on thymic epithelial cells.

The incredible diversity of theoretically more than 10^{15} different human αβTCRs [170] is the result of combinatorial events linking one of multiple variable (V), diversity (D) and joining (J) segments of the TCR loci to produce V-D-J containing β-chains and V-J containing α-chains. Initially, recombination activating gene (RAG) 1 and 2 proteins expressed in CD4/CD8 double-negative (DN) thymocytes introduce DNA breaks at recombination signal sequences (RSS) flanking the V, D and J regions. DNA looping and repair by non-homologous end joining ultimately yield recombined β-chains. Additional junctional diversity is introduced by terminal deoxynucleotidyl transferase (TdT) adding 1-10 nucleotides [171]. Successful recombination of the β-chains allows for the expression of a pre-TCR containing an invariant pre-Tα-chain on the cell surface. At this "β-checkpoint", pre-TCR signaling results in a) "allelic exclusion" that silences the alternative β-chain allele, b) downregulation of RAG1/2 expression and c) several rounds of proliferation and progression to the CD4/CD8 double-positive (DP) stage. Re-expression of RAG proteins in DP thymocytes facilitates the recombination of V and J regions of the α-chain and surface expression of a fully functional TCR. Importantly, failure to successfully recombine the TCR chains may lead to depletion from the thymocyte pool [172,173].

TCR^+ CD4/CD8 DP cells are further selected on MHC molecules presenting self-peptides on thymic epithelial cells to establish tolerance (central tolerance) [174]. This process involves negative selection and elimination of TCRs that bind self-peptide–MHCs too strongly while clones that show low or medium affinities are positively selected and develop into naïve CD4 or CD8 single-positive (SP) T cells. In case of weak or absent interactions, cells undergo "death by neglect" (Starr 2003). As a result, only a fraction of possible V(D)J recombinations and thus unique TCRs actually contribute to the naïve T cell pool in vivo [175]. Nonetheless, it is safe to assume that any foreign peptide will be recognized more or less efficiently due to the cross-reactive nature of most TCRs. While such cross-reactivity is thus a necessity to provide protective immunity, it is also a likely cause for autoimmune disorders [176].

The co-receptors CD4 and CD8 bind MHC class II or class I, respectively, and are crucial for commitment to the CD4 or CD8 SP lineage by recruiting the Src-family kinase Lck to the TCR complex allowing for signal initiation at the ITAMs. As a result, thymocytes bearing MHC class II specific TCRs develop into $CD4^+$ T cells while MHC class I binding results in $CD8^+$ T cell development [177]. Mature but naïve CD4 or CD8 SP T cells exit the thymus into circulation and can further differentiate into effector and memory cells upon activation in secondary lymphoid tissues.

$CD8^+$ T cell differentiation

$CD8^+$ T cells acquire cytotoxic effector molecules and the potential to produce pro-inflammatory cytokines upon further differentiation. Provided the appropriate signals and environmental cues, naïve cells proliferate extensively and generate short-lived effector as well as long-lived memory cells to protect from future insults by same pathogen.

Dendritic cells play a central role in the initial steps of $CD8^+$ T cell activation, proliferation and differentiation. Following infection, exposure of local DCs to PAMPs and pro-inflammatory cytokines such as IFN-γ lead to activation and maturation into a migratory phenotype. Expression of the chemokine receptor CCR7 allows activated DCs to enter lymphatic vessels and home to draining lymph nodes. Importantly, these DCs carry pathogen-derived peptides to lymph nodes where they are cross-presented on MHC class I to naïve CD8 T cells [178]. Besides TCR stimulation (signal 1), naïve T cells require CD28 co-receptor engagement (signal 2) by CD80/86 molecules on the surface of activated DCs as well as pro-inflammatory cytokines (signal 3) such as IL-12 or type I interferons for clonal expansion and differentiation [179–182]. Importantly, lack of co-stimulation results in T cell dysfunction and peripheral tolerance [183,184]. In mouse models of infection, T cell responses usually peak at 7-10 days post infection after which the majority of pathogen-specific cells die in a contraction phase leaving a small portion of memory cells [185]. These will respond rapidly and potently upon re-challenge, quickly giving rise to large numbers of antigen-specific secondary effector cells.

Based on functional and homing potential, mature T cells are categorized into undifferentiated naïve (T_N), short-lived effector (T_{EFF}) as well long-live effector memory (T_{EM}) and central memory (T_{CM}) cells [186,187]. Given the progressive nature of the differentiation process, these subsets have to be appreciated as states within a phenotypic and functional continuum [188,189]. Human $CD8^+$ T_N cells express high levels of the co-receptor CD28, the CD45RA isoform of the protein tyrosine phosphatase CD45, and the lymph node homing receptors CCR7 and CD62L. Specialized to fight pathogens at sites of infection, T_{EFF} cells lack expression of CCR7 and CD62L as well as CD28, but show high expression of perforin and granzymes as well as the potential to produce large amounts of IFN-γ and TNF. Cytotoxicity and cytokine production positively correlate with surface expression of the carbohydrate modification CD57 [190–192], a marker for terminal differentiated or senescence cells with severely impaired proliferative capacity. Divided into central and effector memory populations, memory cells generally express the CD45RO splice variant. T_{CM} cells express CCR7 and CD62L and thus have the potential to enter secondary lymphoid organs. They largely lack perforin and immediate effector function but show increased proliferative capacity compared to T_{EM} cells, which are $CCR7^-CD62L^-$ but often express perforin and rapidly produce IFN-γ upon stimulation [187,193,194]. Truly short-lived, proliferating T_{EFF} as a result of infection can rarely be detected in peripheral blood from healthy donors. Instead, $CD45RA^+$ effector memory cells (T_{EMRA}) lacking CD28 and CCR7 expression are often used to study T_{EFF} biology as they constitute potent killers and cytokine producers [186,193,194].

The STAT5-signaling cytokines IL-2, IL-7 and IL-15 occupy central roles in expansion, homeostasis and survival of $CD8^+$ T cell subsets [195]. Shortly after activation, naïve cells transiently upregulate the high affinity IL-2Rα chain CD25, driving massive clonal expansion of antigen-specific cells by autocrine and $CD4^+$ T cell-derived IL-2 [196]. Prolonged exposure to high concentrations of IL-2 also results in development of short-lived effector at the expense of memory cells [197,198]. Produced by activated DCs and macrophages, IL-15 also aids in clonal effector expansion and is crucial for survival and homeostatic proliferation of memory

cells [199–202]. Similarly, stroma cell-derived IL-7 supports naïve T cell homeostasis and memory T cell survival [202,203].

The precise mechanisms instructing effector versus memory differentiation are still incompletely understood. In mice, IL-12 levels during infection dictate the fate of effector cells by inducing expression of the transcription factor T-bet in a dose dependent manner [204]. High T-bet expression results in development of IL-7R^{low} (CD127) short-lived effector cells (SLECs) while lower T-bet levels give rise to IL-7R^{hi} memory precursor effector cells (MPECs) [204–206]. In line with these observations, IL-12 has been demonstrated to regulate T-bet and Eomes levels after *Listeria monocytogenes* infection in mice to impose effector versus memory fates [207]. Similar to NK cells, both transcription factors are critical for CD8$^+$ T cell differentiation, acquisition of effector functions and survival [105,208]. The transcription factor Blimp-1 also regulates terminal effector cell differentiation and cytotoxicity in CD8 T cells in mice. Blimp-1 deficiency results in impaired Gzmb expression and migration after influenza infection while, cytokine production and memory formation of virus-specific cells are normal [209]. Furthermore, Chang *et al*. proposed a model of effector versus memory fate decision based on asymmetric cell division shortly after initial activation [210]. They found that an unequal distribution of the surface receptors CD25, the signaling molecule PKC-ζ and T-bet during mitosis ultimately directed cells into different fates [210,211].

To cover the energetic and anabolic demands associated with effector and memory states, CD8$^+$ T cells undergo substantial differentiation-dependent metabolic reprogramming [212]. Surprisingly, activated effector cells shift to anaerobic glycolysis for energy production. Although less efficient than catabolic, mitochondrial fatty acid oxidation (FAO) regarding ATP production, intermediates of anaerobic glycolysis are funneled into anabolic processes to provide the macromolecules required for proliferation and effector function [212,213]. In contrast, memory cells have large, fused mitochondria and show overall higher mitochondrial mass allowing for increased FAO and oxidative phosphorylation (OXPHOS) [214]. Important for development and survival, memory cells upregulate fatty acid and triglyceride synthesis as well as lysosomal lipolysis to fuel FAO and OXPHOS [214–216].

1.1.3 Tissue-resident memory T (T_{RM}) cells

T cell recall responses to re-infection are mediated by central and effector memory cell subsets with different functional and migratory properties (see above). The classical models describing memory T cell phenotypes and behavior are mostly based on observations made in peripheral blood and lymphoid tissues from where cells traffic to sites of inflammation in non-lymphoid tissues. This local immunity has thus been considered to be the result of activation, migration and differentiation of patrolling memory cells. Recently, it has become evident that distinct, antigen-specific memory T cells specialized for homing, persistence and homeostatic proliferation in non-lymphoid tissues are generated after infection [217,218]. These tissue-resident T (T_{RM}) cells provide superior local protection against pathogens at barrier tissues such as the skin [219], gut epithelium [220], lung [221,222] and the female reproductive tract [223,224], and have also been characterized in the brain [225] and liver [226,227]. While both CD4$^+$ and CD8$^+$ T_{RM} subsets have been characterized, studies have mostly focused on investigating CD8$^+$ T_{RM} phenotype and function.

The majority of non-recirculating T_{RM} cells in peripheral tissues can be identified by surface expression of CD69, CD103 and CD49a, which promote tissue localization and retention [228–231]. Although CD69 has classically served as a lymphocyte activation marker, it has also

been shown to negatively regulate sphingosine 1-phosphate receptor-1 (S1P1) function to prevent lymphocyte release into circulation [232]. Importantly, transcriptional downregulation of S1P1 is required to seed and establish $CD8^+$ T_{RM} cells in non-lymphoid tissues after infection [233]. As part of the heterodimeric integrin αEβ7, CD103 (integrin αE) binds to E-cadherin [234], which is broadly involved in the formation of adherens junctions between epithelial cells [235]. CD49a, the α1 subunit of the integrin α1β1, mediates adherence to the basement membrane at the epidermal-dermal interface via collagen IV [236,237]. CD49a was first described as very late antigen 1 (VLA-1) on cultured and activated T cells [238,239] and later shown to be expressed on a large proportion of T cells in arthritic joints while being absent on circulating T cells [240]. CD49a has been shown to facilitate tissue retention of lung resident T cell subsets following viral infection in both mice and humans [221,230,241,242].

Importantly, the cytokine TGF-β has a central role in T_{RM} cell formation and retention as it directly controls expression of CD103, CD69 and CD49a [231,243]. In combination with IL-33 and TNF, TGF-β also downregulates expression of the transcription factor KLF2 and its downstream target S1P1, thereby promoting tissue retention [233]. Furthermore, co-expression of the related transcription factors Hobit and Blimp-1 in mice was recently shown to impose the transcriptional program that regulates anatomical localization by repressing genes mediating tissue egress [244]. Lastly, IL-15 has also been demonstrated to be crucial for T_{RM} cell survival and homeostasis [243], similar to its effects on T_{CM} and T_{EM} populations in circulation. In addition, it also been proposed to act as danger signal driving T_{RM} cell activation and effector function that can result in tissue destruction [245].

$CD8^+$ T_{RM} cells in the skin

The human skin serves as important barrier, protecting against injury and infection and is composed of the outermost epidermis and the dermis below. Multiple layers of keratinocytes in combination with melanocytes in the basal layer form the epidermis, which is divided from the dermis by a collagen-rich basement membrane at the epidermal-dermal junction. The dermis consists of connective tissue, fibroblasts and nerve fibers and is drained by blood and lymphatic vessels [246].

Among human tissue samples, skin biopsies are comparatively easy to obtain and human cutaneous T_{RM} cells are thus relatively well studied. In contrast to mice, αβT cells constitute the majority of human skin resident T_{RM} cells in the epidermis and dermis [247]. Interestingly, human prenatal and newborn skin are mostly devoid of αβT_{RM} cells [219,248], which are, similar to mice, likely generated upon pathogen challenge or colonization with commensal bacteria [249]. Indeed, virus-specific skin resident T cells can be detected in active and resolved herpes simplex virus 2 (HSV-2) and varicella zoster virus (VZV) lesions [250–252].

Human skin T_{RM} cells express the inducible carbohydrate modification cutaneous lymphocyte-associated antigen (CLA), which selectively facilitates P-selectin glycoprotein ligand-1 (PSGL-1) binding of E-selectin [253]. While CLA can also be found on a small fraction of circulating T cells, the majority of CLA^+ T cells home to the skin and co-express CCR4 and CCR6 as well as CCR8 and CXCR6 in a subset specific manner [254]. Cutaneous T_{RM} cells display phenotypic properties of T_{EM} cells and largely lack CD45RA and CD62L expression [254,255]. All skin resident human T_{RM} cells express CD69 while $CD103^+$ cells are enriched in the epidermis and display potent effector function but limited proliferative capacity compared to $CD103^-$ cells [219,255]. Importantly, although CD49a can be detected in healthy human skin [221], the distribution of CD49a expression on human T_{RM} subsets and its functional implications are still incompletely mapped (**Figure 2**).

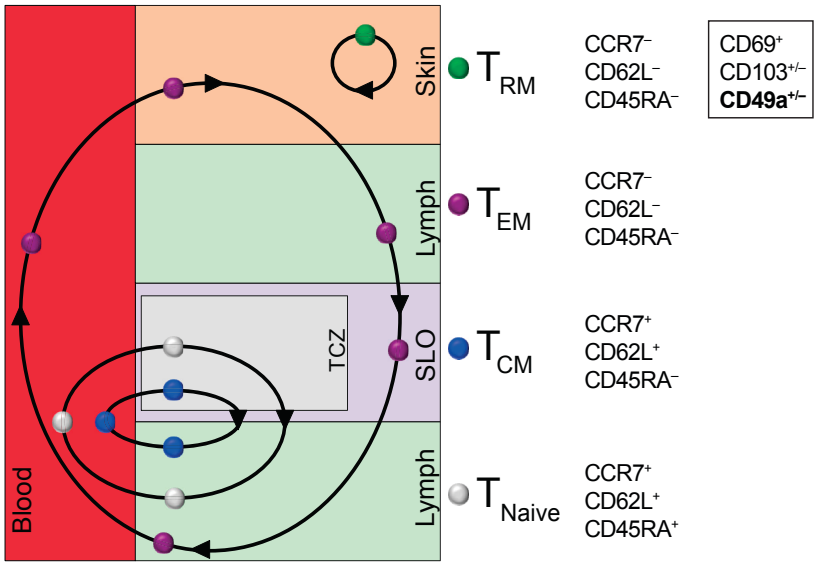

Figure 2. Phenotypic and migratory properties of circulating and skin-resident $CD8^+$ T cell subsets. $CD8^+$ T_{RM} cells in skin largely display a T_{EM} cell phenotype and uniformly express CD69 while expression of CD103 and CD49 are more variable. T_{EM} cells may circulate between lymphoid and non-lypmhoid tissues whereas naïve and T_{CM} cells largely localize to the lymphatic system. TCZ, T cell zone; SLO, secondary lymphoid organ. Adapted from [217].

While local immune responses by T_{RM} contribute to rapid and efficient pathogen containment, dysregulation may result in hyperinflammation and immunopathology as evidenced by spatially confined T cell-driven skin diseases such as psoriasis and vitiligo [256–258]. Psoriasis is characterized by hyperactivation and hyperproliferation of epidermal keratinocytes and cytokine-producing T lymphocytes. Lesions are maintained by cytokines of the IL-23/IL-17 axis and IL-22 producing $CD4^+$ as well as IL-17 producing $CD8^+$ T_{RM} cells are enriched in the epidermis at sites of clinically healed psoriasis, forming a local disease memory [259]. In contrast, cytotoxic T cell-mediated destruction of melanocytes results in localized skin depigmentation observed in vitiligo [260]. $CD8^+$ T cell-derived IFN-γ plays a central role in disease maintenance and progression in human patients and a vitiligo mouse model as it directly induces melanocyte apoptosis [261] and stimulates CXCL10 release promoting T cell recruitment into lesions [262,263].

To better understand the contribution of distinct skin-resident $CD8^+$ T_{RM} subsets to cytokine production and cytotoxicity, we set out to investigate the anatomical localization, transcriptional profiles and functional properties of $CD8^+$ T_{RM} cells in healthy as well as lesional skin from vitiligo and psoriasis patients (**paper V**).

1.2 The cytotoxic machinery and immunodeficiency

Despite the differences in target cell recognition, NK cells and $CD8^+$ T cells largely employ the same cellular machinery to kill target cells by rapid, polarized release of specialized lysosome-like organelles. Key cytotoxic proteins are stored in “secretory lysosomes” or “cytotoxic granules”, part of the endo-lysosomal system that sequentially mature by accumulating effector molecules via fusion of different vesicular structures, although the precise mechanisms are still incompletely understood [2].

Apart from lysosomal proteins and enzymes, cytotoxic granules contain proteins such as perforin and granzymes. Upon release, the multi-domain protein perforin attacks the target cell membrane in a Ca^{2+}-dependent manner followed by pore formation via oligomerization [264,265]. Translated as a precursor in the ER, perforin is sorted into cytotoxic granules via the Golgi apparatus [266]. There, it is proteolytically cleaved by cathepsin L removing a 20 amino acid stretch at the very C-terminus necessary for full biological activity [267,268]. To prevent

activity within the lytic granules, perforin is inhibited by the acidic milieu as well as the proteins serglycin and calreticulin, which bind perforin directly or stabilize the granule membrane, respectively [269,270]. The transmembrane protein lysosome-associated membrane protein 1 (LAMP-1 or CD107a) contributes to protecting the plasma membrane against perforin-mediated attack after granule exocytosis [271]. Importantly, this also allows for flow cytometry-based assessment of cytotoxic granule exocytosis by cell surface staining of CD107a [272,273].

Pore formation in the target cell membrane facilitates entry of other cytotoxic granule constituents, most importantly the serine proteases granzyme A and B. Granzymes reach the lytic granules as pro-enzymes and are processed by the protease cathepsin C and, in case of granzyme B, also cathepsin H [274–278]. In humans, five granzyme genes have been described [279]. While granzyme A and B appear constitutively expressed at high levels in NK cells and CTLs, the expression patterns of granzyme H, K and M are less well defined. Recently, high dimensional flow cytometry data comparing $CD8^+$ T cell and NK cell subsets revealed an increase in expression of granzymes A, B and M throughout terminal differentiation while granzyme K was induced at early but downregulated at later differentiation stages [280]. Once delivered to the cytoplasm of a target cell, granzymes induce apoptosis by caspase-dependent and -independent pathways resulting in mitochondrial damage and generation of reactive oxygen species as well as DNA fragmentation [281]. Experiments in mice suggest central roles for granzyme A and B in target cell killing, as cell death mediated by cells isolated from *GzmA/GzmB* double-deficient mice was strongly reduced and delayed. Furthermore, granzyme B was significantly more efficient inducing apoptosis than granzyme A [282]. Interestingly, granzymes released into the extracellular space also have the potential to regulated pro-inflammatory cytokine production in other cell types [283]. Extracellular granzyme A in particular has been shown to stimulate IL-1β, TNF and IL-6 responses in human and murine monocytes [284].

Due to its destructive potential the release of lytic granules is tightly controlled. After initial contact, a stable but dynamic interaction between effector and susceptible target cell allowing for directed release of lytic granules is formed [285]. Structurally and functionally similar to its neuronal counterpart, this immunological synapse (IS) facilitates efficient killing without affecting bystander cells. The intracellular transport of lytic granules is highly dependent on the microtubule cytoskeleton. Upon contact formation, granules move in minus-end direction towards the microtubule-organizing center (MTOC) while it actively polarizes to the IS [286]. Now in close proximity to the IS, lytic granules are shuttled to the plasma membrane via anterograde transport on microtubules followed by membrane fusion and release of lytic granule contents into the synaptic cleft [287].

Given the importance of lymphocyte cytotoxicity in pathogen defense and immune homeostasis, many processes and components governing lytic granule biology, trafficking and release have been uncovered by studying individuals with primary immunodeficiencies (PIDs) affecting target cell killing. Although disease manifestation and age of onset can differ depending on the type of mutation, genetic defects in lymphocyte cytotoxicity generally result in familial hemophagocytic lymphohistiocytosis (FHL), a life threatening hyperinflammatory condition often triggered by primary herpesviruses infection (Meeths 2014). Mutations in the genes encoding perforin (*PRF1*), Munc13-4 (*UNC13D*), syntaxin-11 (*STX11*) or StxBP2 (*UNC18B*) are causative of FHL type 2-5, respectively [288–291]. Furthermore, mutations in *RAB27A* encoding the small GTPase Rab27a manifest in Griscelli syndrome type 2 (GS2), characterized by degranulation defects in cytotoxic lymphocytes and melanocytes [292]. Cells from individuals with perforin mutations can release lytic granules but fail to deliver granzymes

and induce target cell apoptosis. In contrast, Munc13-4, syntaxin-11, Munc18-2 and Rab27A are involved in granule trafficking, docking to and fusion with the plasma membrane. Munc13-4 is deposited on perforin containing granules upon stimulation with activating receptors [293] and probably regulates interactions between vesicular SNARE (v-SNARE) and target membrane SNARE (t-SNARE) proteins, which provide the physical force for membrane fusion [2]. Rab27a is recruited to lytic granules upon LFA-1 stimulation [293] and facilitates docking to the plasma membrane [285]. Rab27a also interacts with Munc13-4, which has been shown to be crucial for plasma membrane tethering and fusion [294]. Related to classical t-SNAREs, Syntaxin-11 is deposited on the plasma membrane upon TCR stimulation of primary human CTLs [295] and probably mediates the final steps in subsequent membrane fusion and lytic granule exocytosis of cytotoxic lymphocytes as part of the SNARE complex. The SNARE accessory protein Munc18-2 interacts with syntaxin-11 and is vital for syntaxin-11 protein and possibly SNARE complex stability [291] (**Figure 3**).

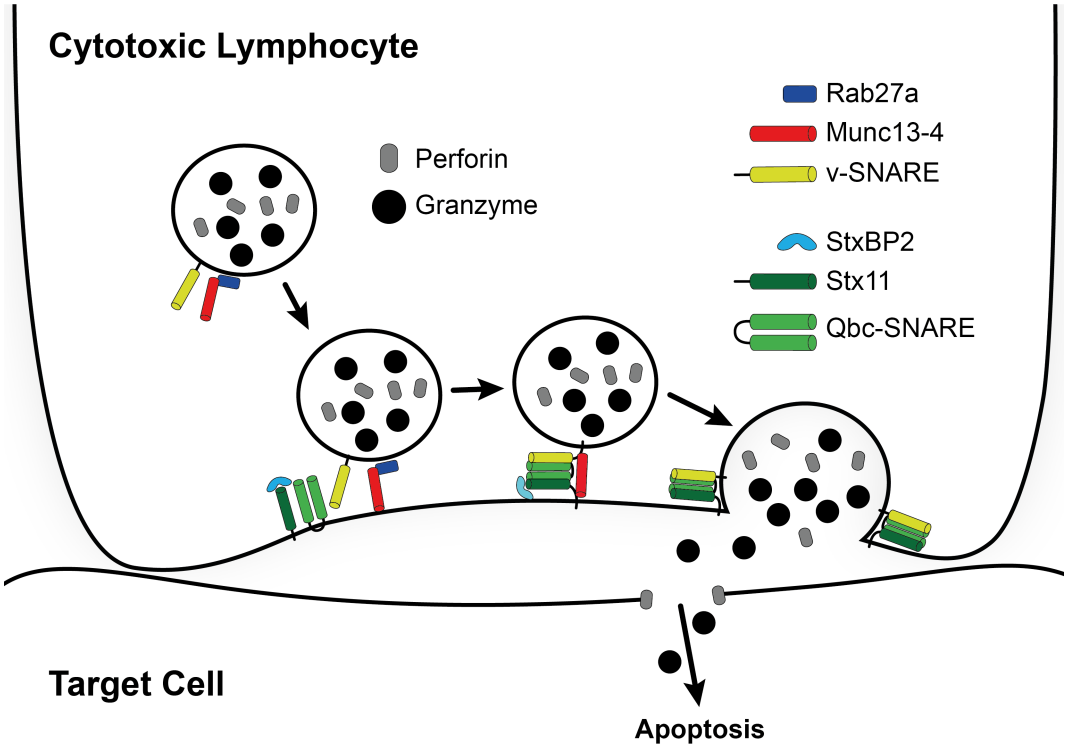

Figure 3. Simplified schematic of factors and events underlying lytic granule exocytosis. Rab27a and Munc13-4 mediate lytic granule trafficking and tethering to the plasma membrane at the immunological synapse (IS). Once in close proximity, SNARE proteins on granules (v-SNARE) and the plasma membrane (Stx11 and a Qbc-SNARE) can interact and form a four-helix bundle that facilitates membrane fusion, leading to release of the granule content and target cell killing. Besides trafficking and membrane tethering, Munc13-4 has also been implicated in promoting SNARE-complex formation. StxBP2 supports SNARE-complex stability and membrane fusion. Adapted from [296].

Importantly, the transcriptional and epigenetic regulation of factors vital for lytic granule exocytosis and their dynamics throughout cytotoxic lymphocyte differentiation are largely unexplored. We recently reported mutations in an evolutionary conserved region of intron 1 of *UNC13D* leading to severely reduced transcription and protein expression. Initially described in European FHL3 patients [297], the c.118-308C>T and the neighboring c.118-307G>A substitution were also highly prevalent in patient cohorts from Asia and North America [298–

300]. In **paper I** we set out to investigate the underlying mechanisms for reduced *UNC13D* transcript levels in these patients, to shed new light on the transcriptional regulation of Munc13-4 expression in cytotoxic lymphocytes and how mutations in non-coding region may cause disease.

1.3 Epigenetic regulation of cytotoxic lymphocyte differentiation

Cellular identity dictating phenotype and function is the result of distinct gene expression profiles that need to be stably maintained or altered upon development and differentiation. While transcriptome analysis can provide insights into differentiation-dependent changes in gene expression, it fails to sufficiently reveal the underlying mechanisms orchestrating such changes. DNA accessibility at gene loci is a major determining factor governing the quality and quantity of gene expression. Nuclear DNA is highly organized and tightly compacted in a chromatin structure of repeating units of nucleosomes comprised of histone octamers. Nucleosome remodeling upon differentiation results in chromatin changes affecting the ability of the transcriptional machinery to access DNA. These changes are mediated by covalent, post-transcriptional modifications of histones and the DNA itself [301–303]. Importantly, while the deposition and removal of these epigenetic marks is in part highly dynamic to allow for processes such as cellular differentiation, they also ensure a stable imprint of cellular identity and function that can be reliably passed on during cell division, features vital to adaptive immunity and immunological memory.

Methylation of DNA occurs at cytosine residues within the context of CpG dinucleotides and methylation at gene promoter regions is usually associated with transcriptional silencing [304]. Maintenance and duplication of DNA methylation during mitosis is mediated by DNA methyltransferase (DNMT) 1 [305] while DNMT3a and DNTM3b function to deposit methyl groups *de novo* [306,304]. DNA demethylation requires further cytosine modification, resulting in failure to duplicate during cell division or base excision repair in non-dividing cells [307].

Post-translational modifications of histones occur at N-terminal lysine residues and can positively or negatively affect transcription. Modifications may influence local charges thereby altering the affinity to DNA, may aid in recruitment of the transcriptional machinery or the assembly of repressive multi-protein complexes [308]. Among the most well studied and understood histone marks described to date are modifications of histone 3 by trimethylation of lysine 4 (H3K4me3) or lysine 27 (H3K27me3) as well as acetylation of lysine 9 (H3K9ac). H3K4me3 is generally associated with transcriptional activation [309] while H3K27me3 marks repressive chromatin. H3K9ac results in increased DNA accessibility and transcription by lowering the affinity to negatively charged DNA. These histone modifications are dynamically regulated by methyltransferases and demethylases as well as histone acetylases (HATs) and –deacetylases (HDACs) [310–313]. Of note, HDACs are often components of repressive complexes bound to methylated DNA, thereby providing a link between different epigenetic marks [314].

Many insights into the changes and dynamics of the epigenetic landscape during cytotoxic lymphocyte differentiation emanate from studies comparing T_N, T_{EFF} and memory (T_{MEM}) $CD8^+$ T cell populations [315,316]. In a genome wide approach, Scharer *et al*. uncovered global changes in DNA methylation comparing T_N and day-8 T_{EFF} cells after LCMV infection in mice. Importantly, promoters, gene bodies and enhancers of effector molecules such as Ifn-γ and Gzmb were demethylated in T_{EFF} compared to T_N cells. In contrast, T_{EFF} cells showed increased methylation at regulatory regions of genes typically expressed in T_N cells such as

Ccr7 and *Tcf7* [317]. Interestingly, transcription factor binding sites also underwent changes throughout differentiation. Putative binding sites for memory associated factors such as Bcl6 were methylated, whereas c-Jun, Nfatc1 and Nfκb binding sites were demethylated in T_{EFF} cells, possibly allowing for rapid binding and initiation of transcription upon activation [317]. Analysis of global methylation and histone marks in human $CD8^+$ T_N, T_{MEM} and T_{EFF} cells revealed stable demethylation and increased histone acetylation at effector molecule loci such as *IFNG*, *TNF*, *PRF1* and *GZMB* as well as loci of the transcription factors *EOMES*, *TBX21* (encoding T-bet) and *PRDM1* (encoding BLIMP-1) in differentiated cells [318,319].

Enrichment of H3K9ac in human $CD8^+$ T_{MEM} compared to T_N cells has been shown to facilitate increased expression of EOMES and its targets perforin and granzyme B [320,321]. In mice, acquisition of H3K9ac at the *Ifng* locus underlies the rapid expression in memory $CD8^+$ T cells [322]. Thus, increased chromatin accessibility at effector gene loci as a result of hyperacetylation imprints memory identity and allows for rapid and robust recall responses.

A hallmark study by Araki *et al*. compared the genome-wide deposition of H3K4me3 and H3K27me3 with mRNA expression levels in resting or short-term activated human peripheral blood $CD8^+$ T_N, T_{EM} and T_{CM} populations [323]. On a global scale, gene expression at rest generally correlated positively with enrichment of H3K4me3 and negatively with H3K27me3 deposition across the gene body. Repressed genes displayed low H3K4me3 but high H3K27me3 levels whereas active sites were characterized by enrichment of H3K4me3 but low levels of H3K27me3. Among the actively transcribed loci in memory cells were genes encoding transcription factors such as EOMES and BLIMP-1 as well as the cytotoxic effector molecules perforin, granzyme A and granzyme B. Poised and bivalent genes required TCR stimulation for expression in memory cells. While poised loci carried histone marks similar to active sites, bivalent loci were enriched for H3K4me3 as well as H3K27me3 and acquired more H3K4me3 after activation, indicating a switch to open chromatin allowing for increased expression at those sites [323]. In line with these observations, experiments in mice later revealed co-deposition of H3K4me3 and H3K27me27 at genes associated with proliferation, replication and differentiation in naïve $CD8^+$ T cells. These bivalent loci rapidly switched to a permissive state by loss of H3K27me3 upon differentiation and included transcription factors such as T-bet, Eomes, Blimp-1 and Nfil3 [324]. In contrast, effector gene loci including *Ifng*, *Prf1* and *Gzmb* acquired H3K4me3 modifications and associated with RNA polymerase II, while losing H3K27me3 marks in differentiated cells [324–326]. Interestingly, T-bet can bind the H3K27-demethylase JMJD3 as well as the H3K4-methylase Set7/9 and recruit those to the *Ifng* locus [327]. These interactions are preserved among all members of the T-box family, highlighting the importance of T-bet and EOMES in chromatin remodeling during cytotoxic lymphocyte development and differentiation.

Lineage specific transcriptional programs are mostly controlled by defined sets of “master” transcription factors that bind open chromatin structures and guide the transcriptional machinery. Lately, the importance of STATs as “pioneering” factors in lineage specification of $CD4^+$ T_H cell subsets has become apparent, linking environmental cytokine cues to epigenetic changes instructing differentiation. In this model, STATs serve to establish open chromatin at lineage specific loci, facilitating access of “master” transcription factors and recruitment of chromatin modifiers for stable epigenetic imprints [328,329]. Given the crucial role of STATs in cytotoxic lymphocyte development and differentiation, similar mechanisms are likely instructing chromatin changes and gene transcription in NK and $CD8^+$ T cells.

While ample data on epigenetic changes during $CD8^+$ T cell differentiation is available, much less is known about the global epigenetic make-up of developing and mature NK cells and how

potential alterations during terminal differentiation processes are regulated to produce the vastly heterogeneous human NK cell repertoire. In **paper II**, we compared global DNA methylation profiles of distinct conventional and adaptive NK cell subsets as well as $CD8^+$ T_N and T_{EMRA} populations to gain insights into the epigenetic patterns that govern their phenotype and function. To uncover the epigenetic basis for human adaptive NK cell diversification we also performed DNA methylation analysis of promoter regions of signaling molecules differentially expressed in conventional compared to adaptive NK cells. Furthermore, to better understand the role of non-coding regions in the development of PIDs and the molecular mechanisms leading to acquisition of cytotoxic potential, we investigated the transcriptional and epigenetic regulation of Munc13-4 expression in **paper I**.

2 RESULTS AND DISCUSSION

In the five papers included in my thesis, I have investigated the differentiation as well as phenotypic and functional diversification of human cytotoxic lymphocyte subsets in health and disease. In **paper I**, we uncovered how the potential for lytic granule exocytosis is acquired and transcriptionally regulated throughout cytotoxic lymphocyte differentiation. **Paper II** describes the epigenetic diversification of differentiated, CMV-associated human adaptive NK cells, while **papers III** and **IV** provide evidence for their longevity. Finally, in **paper V** we determined the phenotypic and functional heterogeneity of $CD8^+$ T_{RM} cells in human skin and how they may contribute to local immunopathology.

2.1 The transcriptional regulation of cytotoxic lymphocyte degranulation

Cytotoxic lymphocytes kill target cells by polarized release of cytolytic granules containing perforin and granzymes [2]. Genetic defects in lymphocyte cytotoxicity result in susceptibility to viral infections and are the underlying cause of familial hemophagocytic lymphohistiocytosis (FHL) as the inability to clear infections as well as to dampen the immune response by killing activated immune cells lead to hyperactivation and hyperinflammation [23]. While the transcriptional regulation of lytic granule constituents such as perforin and granzymes are increasingly well understood [330], the mechanisms governing the expression of components of the exocytic machinery are largely unexplored. Munc13-4 expression is obligatory for lytic granule exocytosis by NK and effector $CD8^+$ T cells, and homozygous mutations in *UNC13D,* encoding Munc13-4, are associated with FHL3 [289]. We previously described non-coding mutations in a conserved region of *UNC13D* intron 1 that resulted in severely decreased mRNA levels in lymphocytes and were causative of substantial proportions of FHL3 cases in Scandinavia, Asia and North America [297–300]. Utilizing theses findings, we set out to study the transcriptional regulation of Munc13-4 in cytotoxic lymphocyte subsets (**paper I**).

Ubiquitous *UNC13D* transcription was previously reported among hematopoietic cells lineages, without specifically addressing transcription in naïve and cytotoxic populations [289]. Surprisingly, we found that Munc13-4 protein was upregulated in differentiated $CD8^+$ effector T cells and $CD56^{dim}$ NK cells relative to immature or naïve subsets as well as non-cytotoxic B and $CD4^+$ T cells. In contrast, the expression of other known mediators of lytic granule exocytosis such as Munc18-2 and syntaxin-11 was similar in all lymphocyte populations or correlated less stringently with cytotoxic potential.

Prediction of transcription factor binding sites revealed that the FHL3-causing *UNC13D* c.118-308C>T mutation was located in a consensus ETS family transcription factor motif flanked by potential STAT sites. ETS factors are important regulators of early lineage commitment in hematopoietic cells and play key roles in lymphocyte development and function [331]. ETS-family members drive expression of perforin in murine cytotoxic T cells [332] and the IL-2 receptor β-chain CD122 in human T cell lines [333]. Moreover, the family member MEF has been shown to facilitate murine NK cell development and perforin expression [334]. Similarly, ETS-1 is critical for early NK cell development by promoting expression of the transcription factors T-bet and ID2 [335,336].

We found that the ETS family member ELF1 directly bound the evolutionary conserved region in intron 1 of *UNC13D.* The role of ELF1 in cytotoxic lymphocyte biology is largely unknown. ELF1-deficient mice show normal NK cell and T cell numbers but severely reduced NKT cell frequencies and cytokine production [337]. T and NK cell function was not addressed in this study.

A high density of binding sites for other cytotoxic lymphocyte associated transcription factors and a conserved transcriptional start site in the intron 1 region suggested a potential role as lymphocyte-specific alternative promoter. We detected the conventional and an additional, shorter *UNC13D* transcript with an alternative first exon that originated from intron 1. Importantly, we found increased levels of the alternative transcript in NK and CD8 T cells opposed to lower levels in B cells and $CD4^+$ T cells from healthy controls. Transcription from the alternative start site was virtually absent in monocytes and all patient cells carrying the homozygous intronic mutations. While the conventional transcript was present in all subsets examined and slightly elevated in monocytes from healthy donors, patient samples uniformly showed residual but severely decreased transcription. Generating isoform specific Munc13-4 antibodies, we have confirmed that the alternative Munc13-4 isoform is preferentially expressed in NK cells and T cells (Galgano, unpublished observations). Our results thus imply a role for the *UNC13D* intron 1 regulatory element as lymphocyte-specific alternative promoter as well as enhancer for the conventional promoter.

Surprisingly, ELF1 protein was broadly expressed in primary lymphocyte populations including B cells and $CD4^+$ T cells. Furthermore transcription from a luciferase reporter construct was independent of ELF1, suggesting that ELF1-binding alone was insufficient to drive increased Munc13-4 expression upon cytotoxic lymphocyte differentiation. We thus speculated that ELF1-binding facilitated recruitment of additional factors to the intronic region responsible for enhanced Munc13-4 expression.

As mentioned above, our analysis of the intronic region predicted putative STAT sites directly up- and downstream (5' and 3') of the ELF1 site. Importantly, IL-12-induced STAT4 signaling plays a critical role in acquisition of cytotoxicity and T-bet expression in mouse $CD8^+$ T cells [180,338]. STAT4 also targets the *PRF1* locus in the human NK cell line NKL [339] as well as the *Ifng* and *IL12RB2* loci during murine and human T_H1 differentiation, respectively [340,341]. We found that STAT4 levels were highest in differentiated $CD8^+$ T cells and NK cells from healthy donors, correlating with Munc13-4 expression and cytotoxic potential. Furthermore, STAT4 bound the 5' but not the 3' STAT site and that binding was at least partially ELF1-dependent. STAT4 Chromatin immunoprecipitation (ChIP) confirmed binding of STAT4 and RNA polymerase II to the intron 1 region in primary NK cells.

STAT4-dependent regulation of murine *Ifng* and human *IL12RB2* loci requires recruitment of BRG1, a component of the SWI-SNF chromatin-remodeling complex, and deposition of active histone marks [340,341]. We found that BRG1 expression mirrored STAT4 and Munc13-4 patterns and was highly induced in cytotoxic $CD8^+$ T cell and NK cells but not naïve or non-cytotoxic primary human lymphocyte subsets. Allele specific PCR after ChIP revealed enrichment of STAT4 and BRG1 at *UNC13D* WT but not mutated intron 1 regions in NK cells from a heterozygous carrier with the *UNC13D* c.118-308C>T mutation. Furthermore, enrichment of the active histone mark H3K27ac was detectable but strongly reduced at the mutated compared to the WT allele. We next confirmed chromatin remodeling and DNA accessibility at the *UNC13D* locus by high-resolution ChIP analysis using antibodies against STAT4, BRG1, H3K4me3 and H3K27ac. We found enrichment of STAT4 and BRG1 binding at intron 1 as well deposition of the active histone marks H3K4me3 and H3K27ac throughout the conventional and intron 1 promoters in primary NK cells and effector $CD8^+$ T cells. Congruently, using formaldehyde-assisted isolation of regulatory elements (FAIRE) analysis [342], we detected enrichment of nucleosome-free regions at the conventional and alternative promoters in NK cells and effector $CD8^+$ T cells compared to naïve T cells and B cells.

As Munc13-4, STAT4 and BRG1 were highly expressed in differentiated $CD8^+$ effector compared to naïve T cells, we investigated the signals and dynamics underlying the induction of those proteins by mimicking differentiation *in vitro*. Surprisingly, only anti-CD3/CD28 stimulation triggered expression of all proteins and STAT4 phosphorylation, albeit comparatively low induction of Munc13-4. BRG1 and STAT4 were induced rapidly, followed by increases in Munc13-4 expression. As STAT4 phosphorylation preceded upregulation of Munc13-4, we speculated that it could promote Munc13-4 induction upon TCR engagement. Indeed, transfection with STAT4 siRNA prior to TCR stimulation prevented Munc13-4 but not BRG1 induction in naïve $CD8^+$ T cells.

In summary, we found that acquisition of cytotoxic potential upon cytotoxic lymphocyte differentiation correlated with upregulation of Munc13-4 expression. FHL3-associated *UNC13D* c.118-308C>T mutations abrogated binding of the transcription factor ELF1 and recruitment of STAT4 to a conserved intronic region that functioned as an enhancer as well as promoter for an alternative Munc13-4 isoform in cytotoxic lymphocytes. Failure to recruit STAT4 prevented BRG1-mediated chromatin remodeling and deposition of active histone marks. Moreover, STAT4 knockdown inhibited TCR-mediated Munc13-4 induction in naïve $CD8^+$ T cells, highlighting the importance of STAT4 in acquisition of lymphocyte cytotoxicity upon differentiation (**Figure 4**).

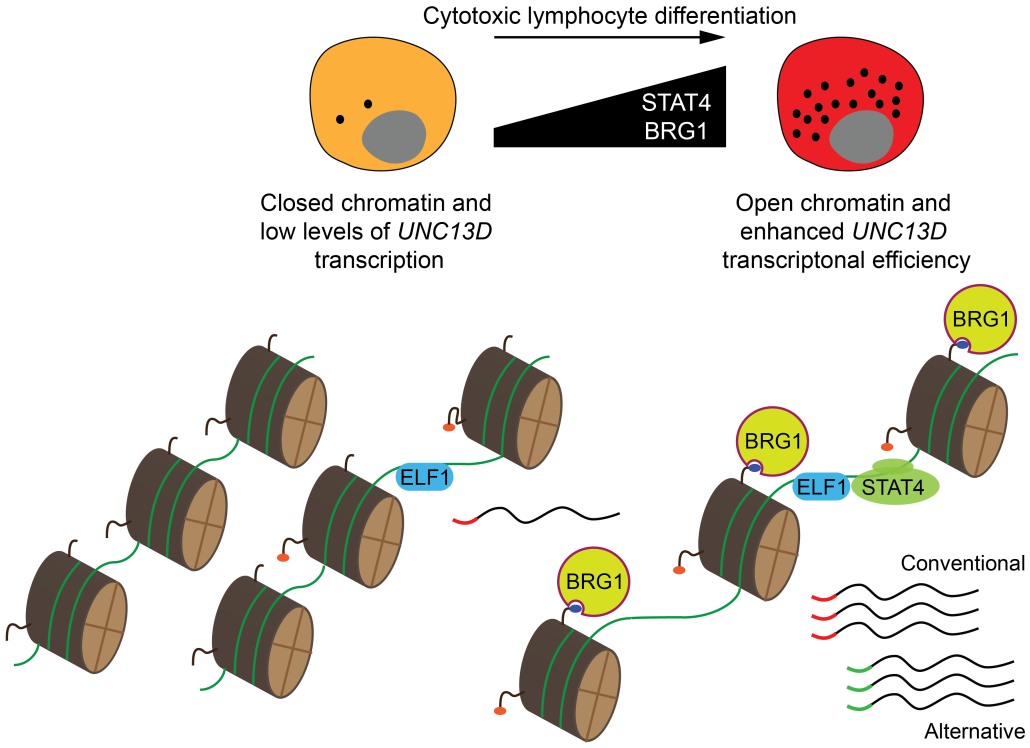

Figure 4. Transcriptional regulation of Munc13-4 expression during cytotoxic lymphocyte differentiation. Binding of ELF1 in intron 1 of *UNC13D* facilitates recruitment of STAT4 and the chromatin remodeler BRG1, both of which are strongly upregulated upon cytotoxic lymphocyte differentiation. This allows for increased DNA accessibility and transcription of the conventional as well as induction of a novel isoform from an alternative intronic *UNC13D* promoter, required for the acquisition of cellular cytotoxicity.

Our results may thus at least partially explain the observed defects in NK cell cytotoxicity in *Stat4*-, *IL12rb1* and *IL12rb2*-deficient mice [343–345] and suggest a central role for STAT4 signaling in lymphocyte cytotoxicity. In line with previous studies reporting TCR-dependent induction of STAT4 in human $CD4^+$ and murine $CD8^+$ T cells [346,347], we detected a rapid increase in protein expression and, curiously, STAT4 phosphorylation after CD3/CD28 stimulation *in vitro.* While this was sufficient to drive Munc13-4 expression in naïve cells, further experiments to determine degranulation capacity and cytotoxic potential following TCR signaling alone would have been tempting. Furthermore, given the role of STAT4 and BRG1 in chromatin remodeling of the *IL12RB2* locus in T_H1 cells [340], our results suggest a requirement for TCR-mediated induction of both proteins to render naïve $CD8^+$ T cells IL-12-responsive and allow for differentiation into potent effector and memory subsets. Therefore, analysis of IL12RB2 expression and IL-12-responsiveness before and after CD3/CD28 stimulation of naïve $CD8^+$ T cells *in vitro* could be interesting.

The mechanisms of TCR-mediated STAT4 phosphorylation remain obscure, but the delayed responses imply an indirect rather than direct involvement of the TCR signaling pathway. A previous report described paracrine/autocrine activation of STATs in primary T cells following TCR and co-receptor engagement which could be blocked by addition of, among others, anti-IFN-γ antibodies [348]. Although naïve $CD8^+$ T cells lack immediate IFN-γ production, a similar mechanism due to "contamination" with recently described stem cell memory T (T_{SCM}) cells may be responsible for our observations. T_{SCM} display a naïve cell surface phenotype but express CD95 and produce IFN-γ, IL-2 and TNF upon activation [349].

Although mechanistic insights are missing, STAT4 single nucleotide polymorphisms (SNPs) are associated with human disease susceptibility. For example, a SNP resulting in reduced STAT4 expression increases the risk for hepatitis B virus-related hepatocellular carcinoma [350]. Furthermore, STAT4 SNPs are linked to complex autoimmune disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome and early onset type 1 diabetes (T1D) [351–353]. Our findings of STAT4-mediated regulation of Munc13-4 expression and cytotoxic lymphocyte function may thus aid in understanding the underlying mechanisms of such conditions and motivate targeted intervention strategies. STAT4 ChIP sequencing will prove useful to identify additional targets in cytotoxic lymphocytes and, in combination with analysis of histone modifications and DNA methylation, provide new insights into how STAT4 epigenetically contributes to cytotoxic lymphocyte identity.

We also demonstrated transcription of a lymphocyte-specific alternative Munc13-4 isoform predicted to be 19 amino acids shorter and encode a unique N-terminal amino acid sequence. Future investigations will be needed to determine the precise roles of the conventional and alternative Munc13-4 isoforms in cytotoxic lymphocyte degranulation. As the *UNC13D* c.118-308C>T mutation completely abolished transcription of the alternative isoform and no disease causing mutations in the first exon of the conventional isoform have been described to date, the alternative isoform is likely to be critical for lymphocyte cytotoxicity. To this end, we have started to examine the roles of the different isoforms in vesicle trafficking and degranulation using isoform-specific monoclonal antibodies.

2.2 The epigenetic signature of adaptive NK cell diversity

Human NK cells are phenotypically and functionally remarkably heterogeneous, but the underlying differentiation processes are only beginning to be understood. Cytokine-producing but poorly cytotoxic $CD56^{bright}$ NK cells mature into $CD56^{dim}$ NK cells that further differentiate

into subsets with diverse cell surface receptor expression and functional potential [354]. Generally, loss of CD62L and acquisition of CD57 as well as inhibitory self-KIRs are associated with increased cytotoxicity and cytokine responses to target cell stimulation [16,118,119]. Following cytomegalovirus infection, subsets of murine and human NK cells have recently been ascribed adaptive immune features such as long-term survival and increased effector functions upon re-stimulation. In humans, these so-called adaptive NK cells often express DAP12-coupled NKG2C or activating KIRs and are educated by inhibitory receptors for self-MHC class I [137,140,145]. CMV-associated adaptive NK cells also lose expression of the ITAM-bearing intracellular signaling adaptor FcεRγ. These cells show severely impaired responses to cancer cell lines but, surprisingly, elevated effector function when stimulated through the Fc receptor CD16 [146,147]. Besides FcεRγ, NK cells express the homologous signaling adaptor CD3ζ, bearing three ITAMs in its cytoplasmic tail compared to only one in FcεRγ [55], which has been suggested to allow for greater responses downstream of CD16. Curiously, in addition to FcεRγ and CD3ζ, NK cells express other pairs of seemingly redundant intracellular signaling molecules including the SYK-family kinases SYK and ZAP-70 [355] and the SAP-family adaptors SAP and EAT-2 [356]. We thus hypothesized that differential expression of signaling molecules may provide a means to modulate signaling properties and tune NK cell activation thresholds and effector functions (**paper II**).

To this end, we assessed protein expression of the homologous transmembrane adaptors FcεRγ and CD3ζ, the SYK family kinases ZAP-70 and SYK as well as the SAP-family adaptors SAP and EAT-2 in $CD56^{dim}$ peripheral blood NK cells. We found that the T cell-associated signaling molecules CD3ζ, ZAP-70 and SAP were uniformly expressed. In contrast, many donors showed downregulation of one or more of the B/myeloid cell-associated signaling proteins FcεRγ, SYK and EAT-2 in subsets of cells. Suggesting a critical role for CMV, loss of signaling protein expression correlated with CMV seropositivity but not age or sex. To determine the influence of acute CMV infection on the generation of NK cells lacking signaling protein we examined a cohort of hematopoietic cell transplant (HCT) recipients of allogeneic umbilical cord blood grafts. We observed development of NK cells lacking signaling protein expression only in recipients that reactivated CMV but not in patients that were CMV seronegative or did not reactive latent virus. Our data thus provided a causative link between acute viral infection and downregulation of signaling proteins in NK cells.

Congruent with an adaptive NK cell phenotype, loss of signaling molecules correlated with NKG2C expression and decreased NKp30 staining. Interestingly, downregulation of FcεRγ correlated strongly with reduced NKp30 surface levels, suggesting that NKp30 couples exclusively to FcεRγ without contribution of CD3ζ for surface retention. In addition to NKG2C, the $FcεRγ^{-}$ compartment was enriched for activating KIRs, implicating a vital role for DAP12-mediated signaling for expansion similar to $Ly49H^{+}$ cells in mice [134]. Generally, frequencies of NKG2A were decreased, while those of inhibitory KIRs elevated within $FcεRγ^{-}$ subsets. Nevertheless, distinct expansions of $FcεRγ^{-}$ cells expressing inhibitory receptors for self-MHC class I in combination with DAP12-coupled NKG2C or activating KIRs could be detected. Importantly, we frequently identified adaptive NK cell subsets lacking expression DAP12-coupled receptors or inhibitory self-KIRs. Thus, we uncovered a previously unappreciated diversity of human adaptive NK cells based on the differential expression of intracellular signaling molecules. Potential functional consequences of such heterogeneity will be discussed.

Intrigued by the observation of tri-modal and seemingly stochastic expression patterns of B/myeloid-associated signaling molecules, we hypothesized that allele-specific, epigenetic silencing of signaling molecule loci contributed to the diversification processes that generate

human adaptive NK cell heterogeneity. Importantly, global DNA methylation changes enforcing memory and effector transcriptional programs have been shown to accompany $CD8^+$ T cell differentiation [317,357]. Consistent with protein expression, we observed extensive promoter DNA methylation in adaptive NK cells and T cells but not canonical NK cells. Expanding on these findings, we perfomed genome-wide DNA methylation analysis. In line with our results on promoter DNA methylation, global adaptive NK cell profiles resembled those of T cells while being markedly different compared to late mature and, even more pronounced, early mature NK cells.

Given that global changes in DNA methylation have been demonstrated to profoundly impact the transcriptional profile during $CD8^+$ T cell differentiation [317], we performed gene expression arrays. As expected, we observed substantial upregulation of *KLRC2* transcripts encoding NKG2C isoform as well as severely reduced *FCER1G* transcription, confirming the quality of our donor selection and sorting strategy. Comparing DNA methylation and gene expression, hypermethylation of probes in proximity to the transcriptional start site (TSS) generally correlated with a reduction in transcript levels and vice versa. It is important to note that due to differing sorting strategies, gene expression and DNA methylation data were derived form different donors using different phenotypic parameters. Intracellular stainings of signaling proteins to stringently define adaptive NK cell subsets still allowed for isolation of genomic DNA and methylation analysis, while this protocol did not support RNA-based gene expression analysis. Using the same donors and subsets would have likely resulted in even better correlation between DNA methylation and transcription. Nevertheless, changes in methylation and transcript levels in adaptive NK cells correlated with protein expression and were consistent previous observations [137,145].

As the transcriptional regulation of human adaptive NK cell differentiation and function were largely unknown, we next focused on differentially expressed transcription factors in adaptive compared to canonical NK cells. Among all transcription factors, transcript levels of the BTB-zinc-finger (BTB-ZF) family member promyelocytic leukemia zinc finger (PLZF), encoded by *ZBTB16*, were the most differentially regulated and strongly reduced in adaptive NK cells. PLZF protein expression was uniformly downregulated in adaptive NK cells marked by loss of FcεRγ, SYK and/or EAT-2. BTB-ZF transcription factors can recruit co-factors for chromatin-remodeling, orchestrate changes in DNA methylation, and regulate lymphoid development and function [358–360]. PLZF ChIP analysis in primary NK cells revealed enrichment at FcεRγ, SYK and EAT-2 but not CD3ζ, ZAP-70 or SAP promoters, suggesting an active role for PLZF in regulation of signaling protein expression. Besides its potential role as global chromatin remodeler, PLZF controls innate programs of NKT and γδT cell lineages and regulates expression of a limited set of genes including IL-12 and IL-18 receptor chains [361–364]. Indeed, *IL12RB2* and *IL18RAP* transcript levels were downregulated in adaptive compared to canonical NK cells, resulting in severely reduced downstream signaling as well as a complete lack of IFN-γ expression after cytokine stimulation. As adaptive NK cells had previously been shown to display reduced responsiveness to IL-12 and IL-18 [145], we now provided a link between loss of the transcription factor PLZF and adaptive NK cell function. Interestingly, IL-12 can also induce expression of the inhibitory receptor NKG2A and impede NKG2C-mediated lysis of HLA-E expressing target cells [365]. It is tempting to speculate that reduced IL-12-responsiveness prevents adaptive NK cell inhibition thus allowing for killing of virus-infected cells.

Zbtb32, another member of the BTB-ZF family, has been shown to facilitate the proliferative burst of murine $Ly49H^+$ memory-like NK cells after MCMV challenge by antagonizing the anti-proliferative effects of Blimp-1 (Beaulieu 2014). Although transcript levels of *ZBTB32* were

equally low in adaptive compared to canonical NK cells, we detected extensive demethylation of probes close to the TSS in adaptive NK cells and effector $CD8^+$ T cells contrasting canonical NK cells and, to a lesser degree, naïve T cells. This may reflect a poised state facilitating the rapid expression of ZBTB32 in adaptive NK cells provided the appropriate stimulus. Surprisingly, recent data demonstrate cooperation between Zbtb32 and Blimp-1 to prevent hyperproliferation of anti-viral cells and promote memory formation in $CD8^+$ T cells after LCMV challenge in mice [366]. The precise roles of ZBTB32 in human adaptive NK cell expansion and differentiation remain to be addressed but are potentially more complex than initially appreciated.

NK cells are also implicated in immunoregulatory killing of activated T cells and antigen-presenting cells to fine-tune adaptive immune responses and maintain immune homeostasis [23,367–370]. Interestingly, experiments in mice suggest that NK cell suppress $CD4^+$ and follicular helper T (T_{FH}) cells thereby inhibiting generation of long-lived virus-specific memory T and B cells as well as production of antiviral antibodies after acute infection [371]. Furthermore, recent data show that upregulation of NK cell-associated genes negatively correlates with antibody responses and protection in a clinical trial investigating the efficacy of malaria vaccines [372]. We found that adaptive NK cells lacking FcεRγ or EAT-2 failed to respond to stimulation with activated autologous T cells. Curiously, SYK single-positive cells remained responsive to activated T cells, implicating a role for NCRs and SLAMFRs in recognition of autologous hematopoietic cells, which is in line with previous reports [87,367]. Thus, the role of adaptive NK cells may shift to surveillance of virus-infected cells as opposed to regulation of other immune cells, which may ultimately promote formation of pathogen-specific memory B and T cell populations.

In summary, we found that human CMV-driven adaptive NK cells lack expression of the B/myeloid-associated signaling molecules FcεRγ, SYK and EAT-2 in a variegated manner. Such silencing correlated with promoter DNA methylation and a global DNA methylation profile that approximated differentiated $CD8^+$ cytotoxic effector T cells. Furthermore, adaptive NK cell differentiation was accompanied by downregulation of the transcription factor PLZF, rendering cells unresponsiveness to innate cytokines (**Figure 5**). Interestingly, adaptive NK cells also failed to respond to autologous activated T cells, suggesting a specialized role in recognition of infected cells.

The exact molecular mechanisms generating adaptive NK cell diversity during differentiation remain to be elucidated, but may be stochastic and asymmetric in nature, similar to what has been observed in $CD8^+$ T cell differentiation from single, naïve precursors [373–376]. Interestingly, a recent study by Freund *et al*. showed that signals from activating receptors were critical for murine NK cell differentiation and acquisition of some Ly49 receptors by regulating the probabilistic switch function of their promoters [377]. Thus, strong activating signals could drive NK cell differentiation in general and adaptive NK cell diversification in particular. While NKG2C engagement by HLA-E on infected cells has been considered the main driver of adaptive NK cells expansion and differentiation, recent data also suggest contributions from other receptors as individuals with homozygous deletions in *KLRC2*, encoding NKG2C, displayed normal frequencies of adaptive NK cells [378]. Adaptive NK cells uniformly express the receptor CD2 and Liu *et al*. demonstrated that CD2 synergized with CD16 stimulation on adaptive but not canonical NK cells. CD2-engagement by its ligand CD58 on CMV-infected, antibody-opsonized target cells could thus drive adaptive NK cell differentiation and diversification independent of NKG2C.

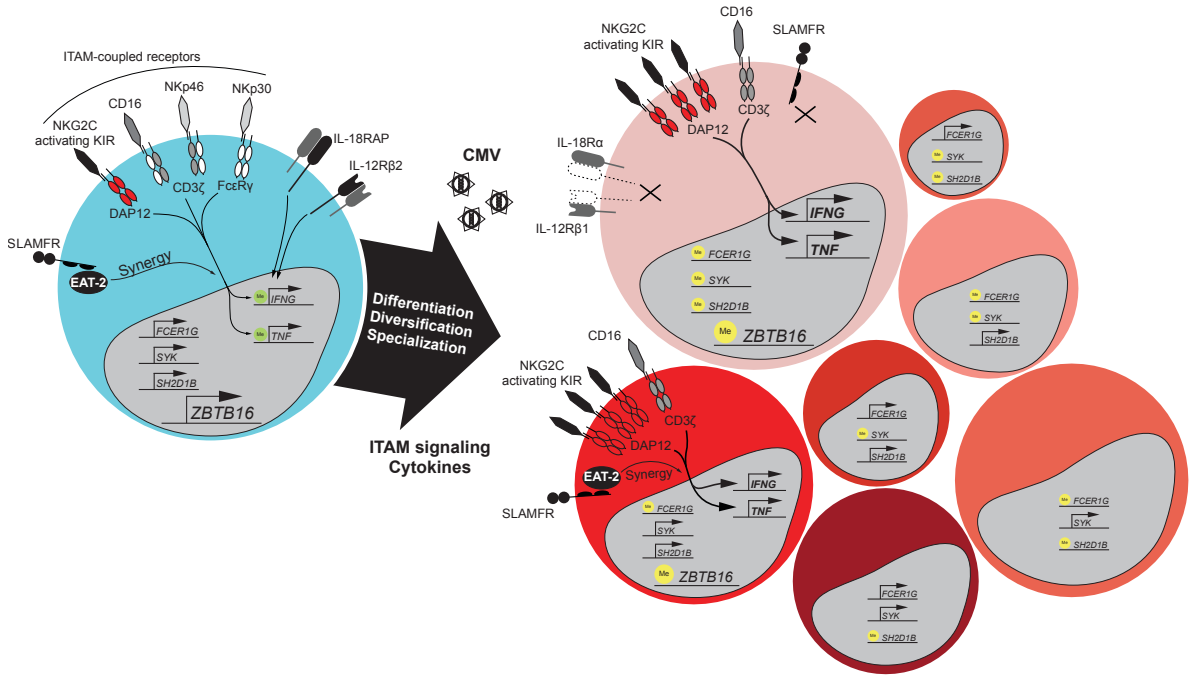

Figure 5. Human adaptive NK cell diversification. CMV infection drives the development of epigenetically heterogeneous adaptive NK cell subsets (red shades; the size of the subsets reflects the relative frequency among adaptive CD56^{dim} NK cells) characterized by DNA methylation-dependent silencing of the transcription factor PLZF (*ZBTB16*) and the signaling proteins FcεRγ, SYK and EAT-2 (*FCER1G*, *SYK* and *SH2D1B*). Downregulation of PLZF results in loss of IL-12 and IL-18 receptor components. Stochastic, allelic methylation of signaling protein promoters generates a diverse adaptive NK cell repertoire with altered target cell specificity by impinging on SLAM family receptor (SLAMFR), NKp30 and NKp46 signaling. DNA hypomethylation at *IFNG* and *TNF* loci allows for increased production of IFN-γ and TNF upon engagement of ITAM-coupled receptors on adaptive NK cells. Adapted from [379].

While canonical NK cell differentiation pathways appear mostly linear and progressive [101], it is still debated whether human adaptive NK cells continue to differentiate and progressively acquire adaptive NK cell features, i.e. loss of additional signaling molecules. Our data suggest a stable phenotype and distribution of the adaptive NK cell repertoire for at least 35 months in a given donor. Any attempts to induce or alter human adaptive NK cell differentiation in our or, to the best of our knowledge any other laboratory, have hitherto been unsuccessful. Furthermore, individuals often display a limited number of stable, “clonal-like” adaptive NK cell expansions. It is thus highly likely that a distinct adaptive NK cell phenotype and repertoire is maintained once generated. How such donor-dependent repertoires are shaped in the first place remains to be shown, but could involve the site and type of CMV-infected target cells, co-infections with other viruses or the genetic background of a given donor. In this regard, twin studies could be insightful to determine the influence of nature and nurture on the development of distinct adaptive NK cell subsets. In addition, the underlying causes determining the size of the adaptive NK cell pool and why some individuals lack such cells despite CMV exposure are of great interest. Here, correlating the CMV-specific adaptive immune response with the adaptive NK cell frequency after primary infection could provide clues regarding the broad distribution observed in the healthy cohorts investigated to date.

In line with previous observations describing DNA demethylation at the *INFG* locus in NKG2C^{+} NK cells [142], we detected extensive hypomethylation at *IFNG* as well as *TNF* loci in adaptive NK cells, which is likely to contribute to increased transcription and cytokine production. NK cell education by inhibitory receptors for self-MHC class I [15–17] as well as decreased expression

[380] or engagement [381] of activating receptors have been linked to increased NK cell function. We speculate that loss of intracellular signaling protein expression may have similar effects by reducing tonic signaling from activating receptors resulting in NK cell desensitization through epigenetic remodeling at and increased accessibility of cytokine loci.

2.3 Evidence for human adaptive NK cell longevity

Adaptive or memory-like NK cell subsets expand and persist after CMV infection in mice and man [134,137,140,141,143,144]. In **paper II**, we found that the size and phenotype of a donor-specific adaptive NK cell repertoire at steady-state, defined by expression patterns of surface receptors and intracellular signaling proteins, remains remarkably stable for up to 35 months. Nevertheless, it is still unclear whether human adaptive NK cells are continuously replenished by cells differentiating from hematopoietic progenitors and immature NK cells or undergo slow, homeostatic turnover similar to B and T cell memory populations [382,383]. To address this issue, we utilized samples from patients with bone marrow disorders associated with *GATA2* haploinsufficiency (**paper III**) or acquired somatic *PIGA* mutations in hematopoietic stem and progenitor cells (HSPCs) underlying paroxysmal nocturnal hemoglobinuria (PNH) (**paper IV**).

The transcription factor GATA-2, encoded by the *GATA2* gene, is required for HSPC survival and proliferation [153,154]. In humans, heterozygous loss-of-function mutations are associated with progressive loss of monocytes, DCs, B cells and NK cells resulting in immunodeficiency and increased susceptibility to certain infections [155–158]. In some patients, subsets of differentiated NK cells accumulate, which led us to hypothesize that adaptive NK cells could persist in the absence of HPSCs and thus prove their longevity or potential for homeostatic turn-over, independent of constitutive cellular output from the bone marrow. Indeed, in a cohort of patients with heterozygous *GATA2* mutations that retained sizeable frequencies of peripheral blood NK cells, we found that these cells almost uniformly displayed phenotypic and functional characteristics of adaptive NK cells as defined in **paper II**. Previously, Mace *et al*. reported GATA-2 expression in $CD56^{bright}$ but not $CD56^{dim}$ NK cells, linking *GATA2* haploinsufficiency to the specific loss of $CD56^{bright}$ NK cells in patients [163]. Using multicolor flow cytometry, we were not able to recapitulate those findings as GATA-2 expression was confined to $CD34^+$ HSPCs in bone marrow and blood. Instead, we found that $CD3^-CD14^-CD16^-CD20^-CD56^-$ lineage-negative (Lin^-) cells from patients with heterozygous *GATA2* mutations displayed a severely reduced capacity to generate mature NK cells in an *ex vivo* NK cell expansion and differentiation protocol. Examination of Lin^- cells revealed significantly diminished frequencies of NK cell progenitors in *GATA2* patients compared to healthy donors, supporting our notion that *GATA2* haploinsufficiency results in attrition of the HSPC pool and gradual loss of constitutively generated, short-lived canonical but not long-lived adaptive NK cells (**Figure 6**).

In contrast to individuals with *GATA2* deficiency, PNH patients with acquired X-linked *PIGA* mutations in HSPCs have not been reported to develop immunodeficiency, but generally present with decreased leukocyte counts compared to healthy donors [384]. Importantly, despite a reduction in total NK cells numbers, the distribution and functional responses of NK cell subsets appear unaffected [385]. Due to a defect in the GPI-synthesis pathway, patients lack expression of GPI-anchored proteins on the cell surface of mature progeny arising from mutated HSPC clones. Loss of the GPI-anchored proteins CD55 and CD59 on red blood cells (RBCs) results in complement-mediated membrane attack and lysis, a main characteristic of disease presentation in PNH [386]. After years of stable mosaicism allowing for the detection of

GPI$^+$ and GPI$^-$ leukocytes subsets in the periphery, hematopoiesis may be progressively dominated by HSPC clones with somatic *PIGA* mutations. While B and T cells mostly remain GPI$^+$, neutrophils and RBCs in particular completely convert to a GPI$^-$ phenotype, likely reflecting the comparatively short half-life and rapid renewal from HSPCs. Similarly, CD56^{bright} NK cells in some patients have been reported to mostly comprise GPI$^-$ cells, while CD56^{dim} NK cells often display a more even distribution of GPI$^+$ and GPI$^-$ cells [387]. Given that peripheral blood NK cells have an estimated half-life of roughly two weeks [166] and would be readily replenished from GPI$^-$ progenitors, we speculated that long-lived adaptive NK cells largely remain GPI$^+$. We thus assessed the contribution of GPI$^+$ and GPI$^-$ cells to canonical and adaptive NK cell subsets in a cohort of PNH patients with varying degrees of chimerism in the neutrophil compartment (**paper IV).** Somewhat expected and in line with previous observations [388], frequencies of GPI$^-$ CD56^{bright} NK cells strongly correlated with the fraction of GPI$^-$ neutrophils. In contrast, CD56^{dim} NK cells contained overall fewer GPI$^-$ cells and only a minor fraction of B and T cells displayed a GPI$^-$ phenotype. Importantly, we found that the vast majority of CD56^{dim} adaptive NK cells retained expression of GPI-anchors even in patients with nearly complete conversion of neutrophils and CD56^{bright} NK cells, suggesting homeostatic maintenance of adaptive NK cells for more than ten years independent of HPSCs. In contrast, canonical CD56^{dim} NK cells contained mostly GPI$^-$ cells, indicating constant renewal from *PIGA* mutated progenitors (**Figure 7**).

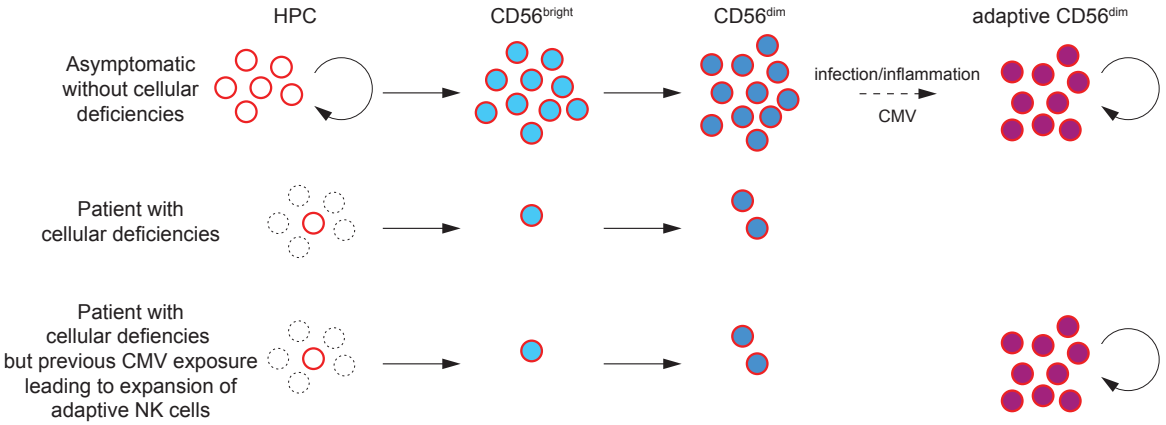

Figure 6. Proposed model for the accumulation of adaptive NK cells in patients with heterozygous *GATA2* mutations. Hematopoietic progenitor cells (HPCs) support NK cell development and potentially CMV-associated adaptive NK cell differentiation and expansion in asymptomatic carriers. Gradual loss of HPCs due to GATA-2 haploinsufficiency abrogates the development of short-lived CD56^{bright} and canonical CD56^{dim} NK cells. However, patients that generated adaptive NK cells prior to HPC attrition may retain high frequencies of potentially long-lived adaptive NK cells with the propensity for homeostatic self-renewal.

Therefore, utilizing peripheral blood samples from two cohorts of patients with bone marrow disorders, we demonstrate that human adaptive NK cells mirror memory T and B cells and are likely maintained by HSPC-independent processes. The precise molecular mechanisms underlying adaptive NK cell survival or homeostasis are unknown, but are at least partially the result of increased expression of anti-apoptotic molecules such as Bcl-2, which is induced in FcεRγ$^-$ adaptive NK cells [147] and has been demonstrated to mediate formation and survival of memory B and T cells [389–391]. Bcl-2 expression responsible for CD8$^+$ T cell memory

survival and homeostasis is largely driven by IL-15 [392,393], which also supports survival of murine NK cells by inactivating the pro-apoptotic transcription factor FoxO3a [394]. Surprisingly, we have detected enhanced IL-15 sensitivity and overall higher levels of phosphorylated STAT5 after IL-15 stimulation in adaptive compared to canonical NK cells (unpublished observation). Low IL-15 concentrations could thus potentially support long-term survival of adaptive but not canonical NK cell. Moreover, PLZF has curiously been shown to predispose human NKT and MAIT cells to apoptosis as a result of increased expression of activated caspases [395]. Loss of PLZF expression could therefore promote adaptive NK cell longevity while canonical NK cells undergo continuous renewal from HSPCs. Furthermore, the mitophagy-mediated removal of dysfunctional mitochondria during the antiviral response has recently been shown to promote the generation of memory NK cells in mice [396] but a similar mechanism has still to be demonstrated in man. Overall, future experiments addressing the regulation of pro- and anti-apoptotic pathways will be needed to provide further insights into the mechanisms governing adaptive NK cell homeostasis and survival. This could aid in the development of strategies for efficacious NK cell-based cancer immunotherapy.

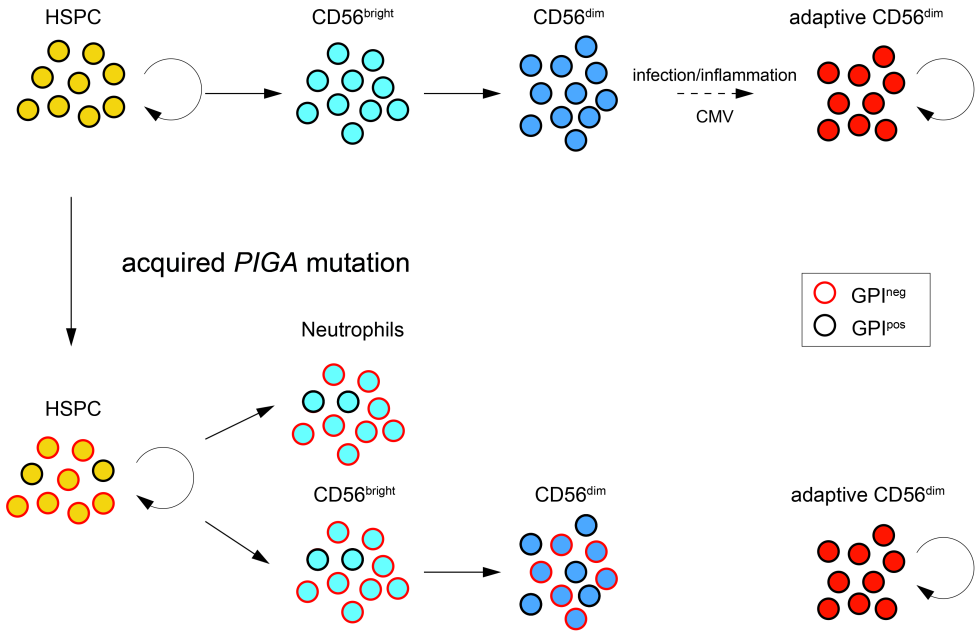

Figure 7. Proposed mechanism underlying the persistence of GPI^{+} adaptive NK cell in PNH. Patients may experience CMV-driven differentiation and expansion of GPI^{+} adaptive NK cells before the acquisition of *PIGA* mutations in hematopoietic stem and progenitor cells (HSPCs). After mutation of *PIGA*, GPI^{-} HSPCs progressively contribute to the development of short-lived mature hematopoietic cells including neutrophils as well as CD56^{bright} and canonical CD56^{dim} NK cells. However, adaptive NK cells remain GPI^{+}, supporting the notion of HSPC-independent maintenance due to longevity and/or homeostatic turnover.

2.4 A dichotomy of CD8^{+} T_{RM} cells in human skin

While **papers I-IV** investigated general concepts of lymphocyte cytotoxicity as well as human adaptive NK cell diversity and longevity, we studied the heterogeneity of CD8^{+} T cell phenotype and function in non-lymphoid tissue in **paper V**. Exposure to pathogens and commensal bacteria drive formation of T_{RM} populations providing local immunity at barrier sites such as

skin, gut, lung and the female reproductive tract [218,256,397]. T_{RM} cells get rapidly activated to combat recurrent infections but may contribute to local immunopathology as evidenced by focal skin diseases such as psoriasis and vitiligo [256–258]. Driven by cytokines of the IL-23/IL-17 axis, psoriasis is characterized by hyperinflammation and hyperproliferation of epidermal keratinocytes [257], while vitiligo lesions display hypopigmentation caused by immune-mediated loss of melanocytes [258]. Given the differences in the underlying mechanisms and disease presentation, it is likely that functionally different T_{RM} subsets contribute to psoriasis and vitiligo. Functional diversity of cutaneous T_{RM} cells with regards to cytokine expression has been reported but phenotypic markers reflecting such diversity have not been established [219]. T_{RM} can be distinguished from circulating T cell subsets by expression of CD103 and CD69, which mediate tissue-retention by binding epithelial E-cadherin and antagonizing S1P1 function, respectively [228,229,233]. Interestingly, recurrent viral infections drive the accumulation of anti-viral T_{RM} cells expressing CD49a, the α-subunit of the $\alpha_1\beta_1$ integrin, in non-lymphoid tissues [230,398]. CD49a binds collagen IV, which is enriched in the basement membrane separating dermis and epidermis [237], and thus likely aids in positioning $CD49^+$ T_{RM} at the dermal-epidermal interface. In healthy human skin, CD49a expression is variable [221], which led us to speculate that CD49a might indicate functionally distinct T_{RM} subsets with potentially specific contributions to human skin diseases. We thus examined the anatomical localization, transcriptional profiles and functional properties of $CD8^+$ T_{RM} cells in human skin from healthy donors as well as lesional skin from patients with psoriasis or vitiligo (**paper V**).

In healthy human skin, CD49a expression was largely restricted to epidermal $CD8^+$ T_{RM} cells co-expressing the T_{RM} markers CD103 and CD69. Reflecting a high degree of variation of the T_{RM} cell compartment, frequencies of $CD49a^+$ cells were variable among and even within donors when comparing different sites. Confocal microscopy revealed that epidermal $CD8^+$ T_{RM} cells localized to the basal membrane irrespective of CD49a expression. We next assessed the clonal relationships between tissue-resident and circulating effector memory $CD8^+$ T cells in five donors by DNA sequencing of the TCRβ complementary-determining region 3 (CDR3). We found that epidermal $CD49a^+$ $CD8^+$ T_{RM} cells in three donors were dominated by a single but different Vβ chain. Furthermore, T cell subsets isolated from peripheral blood and the dermis as well as $CD49a^-$ epidermal T cells generally displayed greater CDR3 diversity compared to $CD49a^+$ epidermal T_{RM} cells. Importantly, the ten most abundant TCR clones in epidermal $CD49a^+$ T_{RM} were only minimally shared with all other subsets, thus indicating a unique, clonally enriched population of $CD8^+CD69^+CD103^+CD49a^+$ T_{RM} population in healthy human skin. The observed variability in size and clonal distribution of this population is likely reflective of the local history of pathogen exposure resulting in dominant clonal expansion.

We next performed transcriptional profiling by RNA sequencing to determine additional phenotypic and potential functional differences between epidermal $CD103^+CD49a^+$ and $CD103^+CD49a^-$ $CD8^+$ T_{RM} cells. Principal component analysis revealed that transcriptional profiles of epidermal populations clustered separately from dermal as well as peripheral blood T cells and, in addition, showed distinct clusters of epidermal $CD49a^+$ and $CD49a^-$ $CD8^+$ T_{RM} cells. Interestingly, genes indicating cytotoxic function as well as anti-viral responses, lymphocyte activation and chemotaxis were enriched among the 92 differentially expressed genes comparing epidermal $CD49a^+$ and $CD49a^-$ $CD8^+$ T_{RM} cells. Specifically, $CD49a^+$ cells showed increased transcription of lytic granule components such as *PRF1*, *GZMB*, *GZMH*, *GZMK*, *GNLY* and *NKG7* as well as *IFNG* and *CXCR3*. In contrast, $CD49a^+$ cells displayed significantly decreased transcription of genes associated with IL-17 production such as *IL17F*, *RORC*, *IL23R and CCR6* in comparison to $CD49a^-$ cells. Thus, transcriptional profiles implicated distinct functional specializations of epidermal $CD49a^+$ and $CD49a^-$ $CD8^+$ T_{RM} cells,

where CD49a$^+$ cells engage in type I and cytotoxic responses, while CD49a$^-$ cells contribute to IL-17-mediated inflammation.

Surprisingly, although *PRF1* and *GZMB* transcripts were readily detectable, freshly isolated, resting epidermal CD103$^+$CD49a$^+$ CD8$^+$ T_{RM} lacked perforin and granzyme B protein expression. This was reminiscent of murine NK cells containing abundant *Prf1* and *Gzmb* mRNA but require stimulation with STAT5 cytokines IL-2 or IL-15 for protein expression [399]. Furthermore, IL-15 was recently proposed to act as danger signal driving T_{RM} cell effector function for tissue destruction [245] and facilitates cytotoxic effector differentiation *in vitro* when produced and presented by epidermal Langerhans cells [400]. Indeed, stimulation with IL-2 and IL-15 but not other cytokines associated with skin inflammation rapidly induced perforin and granzyme B protein expression specifically in CD49a$^+$ T_{RM} cells. In line with these observations, CD49a$^+$ but not CD49a$^-$ T_{RM} cells acquired strong cytotoxic potential and killed target cells in a TCR-dependent manner after priming with IL-15. Of note, prolonged TCR stimulation alone induced perforin and granzyme B protein expression and displayed additive effects when combined with IL-15 priming. As T_{RM} cells are not confined to the skin and populate other barrier tissues, we examined CD49a as well as perforin and granzyme B expression in T_{RM} cells isolated from gut and cervix. CD49a$^+$ CD8$^+$ T_{RM} cells were readily detectable in gut and cervix and, surprisingly, showed significant perforin and granzyme B expression at rest, which was further augmented by IL-15 priming. The generally elevated baseline expression of perforin and granzyme B in these tissues may reflect an increased exposure to pathogens or commensal bacteria compared to the skin. We thus found that CD49a expression in human T_{RM} cells correlates with cytotoxic potential. Specifically, CD103$^+$CD49a$^+$ CD8$^+$ T_{RM} cells in healthy human skin are poised for cytotoxicity and can kill target cells after priming with IL-2 or IL-15 alone or, even more efficiently, in combination with antigen. Among the potential local sources of IL-2 or IL-15 are activated T cells or keratinocytes and epidermal Langerhans cells [400,401], respectively.

Besides perforin-mediated target cell killing, CD8$^+$ T cells produce pro-inflammatory cytokines to fight invading pathogens. At barriers sites, T cell-derived IFN-γ and IL-17 elicit anti-viral and anti-fungal responses, respectively. Given the elevated *IFNG* transcript levels in epidermal CD49a$^+$ CD8$^+$ T_{RM} cells and the increased transcription of *RORC*, associated with IL-17 production, in epidermal CD49a$^-$ CD8$^+$ T_{RM} cells, we speculated that CD49a$^+$ expression might separate T_{RM} subsets with distinct cytokine profiles in human skin. Indeed, following activation we found preferential expression of IFN-γ in epidermal CD49a$^+$ cells, while IL-17 production was largely confined to epidermal CD49a$^-$ cells. IL-15 priming resulted in a significant increase in production of both cytokines and even promoted IFN-γ production in epidermal CD49a$^-$ CD8$^+$ T_{RM} cells. Dermal CD8$^+$ T_{RM} populations produced only minimal amounts of IL-17 while IFN-γ was more broadly expressed in sizeable fractions of dermal CD103$^+$ and CD103$^-$ subsets. As IFN-γ and IL-17 are implicated in the focal skin diseases vitiligo and psoriasis, respectively, we next determined the expression of CD49a, perforin and granzyme B as well as IFN-γ and IL-17 in resting CD8$^+$ T_{RM} cells from skin lesions. Frequencies of epidermal and even dermal CD103$^+$ CD8$^+$ T_{RM} expressing CD49a were significantly increased in vitiligo but not psoriasis compared to healthy donors. Importantly, significant fractions of dermal and epidermal CD49$^+$ cells in vitiligo readily expressed perforin and granzyme B, indicating previous priming by cytokines and/or TCR stimulation. This notion was further supported by an increased frequency of total epidermal CD8$^+$CD103$^+$ T_{RM} cells with the potential to produce IFN-γ in vitiligo compared to psoriasis lesions or healthy skin. Moreover, although epidermal CD49a$^-$ cells readily produced IFN-γ, frequencies of IFN-γ$^+$ cells were significantly higher among CD49a$^+$ cells. In contrast, epidermal CD8$^+$CD103$^+$ T_{RM} cells in psoriasis were primed for

IL-17 production and frequencies of IL-17$^+$ cells after activation were significantly elevated in CD49a$^-$ compared to CD49a$^+$ cells.

In summary, we uncovered a functional specialization of human skin CD8$^+$ T_{RM} cells in regards to cytotoxicity and cytokine production, which was preserved and enforced in the focal skin diseases vitiligo and psoriasis. In healthy skin, epidermal CD8$^+$CD103$^+$CD49a$^+$ T_{RM} cells were poised for IFN-γ production and cytotoxicity, and rapidly induced expression of perforin and granzyme B upon priming with IL-2 or IL-15, facilitating target cell killing. Importantly, primed CD8$^+$CD103$^+$CD49a$^+$ T_{RM} cells accumulated in the dermis and epidermis of vitiligo lesions where they likely mediate the destruction of melanocytes. In contrast, epidermal CD8$^+$CD103$^+$CD49a$^-$ T_{RM} cells excelled at IL-17 production and were enriched in psoriasis lesions, probably contributing to the local inflammatory milieu driving keratinocyte hyperactivation and –proliferation (**Figure 8**).

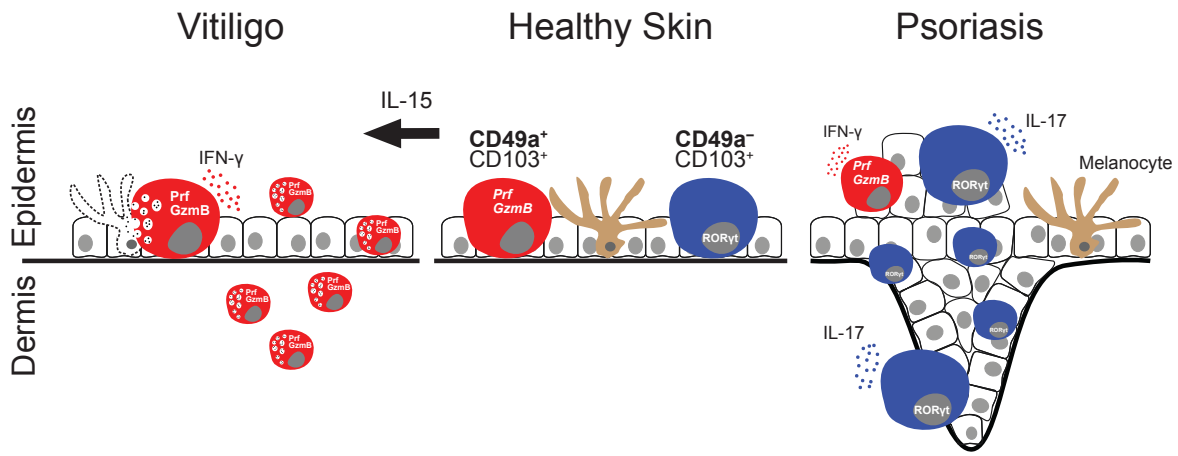

Figure 8. CD49a expression defines functionally distinct CD8$^+$ T_{RM} cells in healthy skin as well as vitiligo and psoriasis lesions. In healthy skin, CD49a$^+$ cells (red) poised for cytotoxicity and IFN-γ production localize to the epidermis, while epidermal CD49a$^-$ cells (blue) possess the potential to release IL-17. In vitiligo, primed CD49a$^+$ cells readily expressing perforin and granzyme B accumulate in the epidermis and dermis of lesional skin where they contribute to melanocyte destruction by cellular cytotoxicity and IFN-γ release. In contrast, psoriasis lesions are enriched for IL-17 producing CD49a$^-$ cells but also contain IFN-γ producers, creating a hyperinflammatory milieu.

Barrier tissues represent entry sites for invading pathogens in general and viruses in particular. Common pathogenic viruses such as herpes simplex viruses (HSVs), human papilloma viruses (HPVs) and varicella zoster virus (VZV) target the human skin and establish latent or chronic infections [402,403]. Therefore, local deposition of CD8$^+$ T_{RM} cells with the capacity to rapidly induce anti-viral responses by IFN-γ production and lysis of infected cells represents an effective defense strategy against re-infection and, importantly, reactivation. As previously shown for murine epithelia [230,231,398], we found that cells with such function in healthy human skin localize to the epidermis and express CD49a, which probably serves to position cells at the basal membrane dividing dermis and epidermis where they can target infected keratinocytes. Epidermal CD49a$^+$ cells will probably also aid in recruitment of circulating immune cells to fight infections, either directly by expressing chemokines such as MIP-1β, similar to circulating memory and effector CD8$^+$ T cells, or indirectly by IFN-γ-induced chemokine production by local immune and non-immune cells [263,404].

Interestingly, in contrast to circulating effector memory subsets, epidermal CD49a$^+$ CD8$^+$ T_{RM} cells lacked perforin and granzyme B expression and relied on priming by IL-2 or IL-15 for cellular cytotoxicity. IL-15 in particular has been shown to be crucial for T_{RM} cell development and homeostasis [243,405], and has recently been suggested to function as danger signal for tissue-resident T cells [245]. Besides epidermal Langerhans cells [400], epidermal keratinocytes produce IL-15, which may be boosted by IFN-γ [406]. Surprisingly, IL-15 mRNA can be readily detected in freshly isolated epidermal keratinocytes and Langerhans cells [407]. Thus, low IL-15 levels could support T_{RM} cell homeostasis at steady-state without licensing CD49a$^+$ CD8$^+$ T_{RM} cells for cytotoxicity. Rapid IFN-γ production by CD49a$^+$ CD8$^+$ T_{RM} cells upon infection could ultimately increase IL-15 expression and tip the balance towards cytotoxic effector function and tissue destruction. Given that IL-15 also promoted IL-17 production by epidermal CD49a$^-$ CD8$^+$ T_{RM} cells, this pathway may represent an attractive target for the treatment of cutaneous autoimmune conditions associated with aberrant T_{RM} cell function. Indeed, inhibition of JAK1 and JAK3 by the small molecule tofacitinib has recently shown promising results for the treatment of vitiligo in humans [408]. Furthermore, antibody-mediated blockade of the IL-15 receptor complex in mouse models facilitated the resolution of psoriasis [409] and prevented hair loss associated with alopecia areata [410]. On the other hand, administration of recombinant IL-15, IL-15/IL-15Rα complexes or IL-15 agonists have shown significant potential in the treatment of cancers by inducing proliferation, redistribution and enhanced effector function of cytotoxic lymphocytes [411,412]. Moreover, IL-15 has been shown to permit TCR-independent activation and cytotoxicity of CD8$^+$ T cells in gut epithelium of patients with active celiac disease [413,414]. Cytotoxicity resulting in tissue destruction was mediated by the activating receptor NKG2D, which binds to stress-induced ligands such as MICA/B and ULBPs in humans. Importantly, NKG2D ligands are also expressed early during malignant transformation and can be found on human melanoma cells [415,416]. In mice, skin-resident γδT cells have been shown to protect from development of cutaneous malignancy after exposure to carcinogens in an NKG2D-dependent manner [417]. Therefore, IL-15 might broadly license epidermal CD49a$^+$ CD8$^+$ T_{RM} cells to provide a first line of defense against tumor development by NKG2D-mediated cytotoxicity.

Although the precise signals driving the differentiation of distinct CD8$^+$ T_{RM} effector subsets from a common naïve precursor remain to be elucidated, the cytokine environment will be of central importance. Additionally, a certain degree of plasticity among T_{RM} cells, allowing to transition between different types of effector functions *in situ*, may confer greater flexibility for the defense against a variety of different pathogens and threats. A better understanding of the signals governing differentiation and effector functions of diverse, specialized T_{RM} subsets could ultimately aid in developing strategies for the treatment of autoimmune disorders or infections, and may potentially improve vaccination efficacy through optimization of memory formation [418,419].

3 CONCLUDING REMARKS

NK cells and $CD8^+$ cytotoxic T cells constitute the major subsets of human cytotoxic lymphocytes and protect against viral infections and cancer development. They can kill aberrant cells by directed release of granules containing cytotoxic proteins and produce soluble factors to instruct the immune response. Importantly, cytotoxic lymphocyte dysfunction may result in disease. Defects in cytotoxicity cause severe susceptibility to viruses and hyperinflammation due to an inability to clear infected cells as well as activated immune cells. On the other hand, unrestrained activation may lead to destruction of healthy tissue and autoimmunity. In this thesis, I have examined different aspects of human cytotoxic lymphocyte differentiation to expand our understanding of i) the mechanisms underlying the acquisition of cytotoxicity, ii) the phenotypic and functional diversification in response to cytomegalovirus as well as iii) the contribution of distinct of skin-resident T cell subsets to tailored immune responses in health and disease.

In **paper I**, we found that expression of Munc13-4, a factor crucial for lytic granule exocytosis, was upregulated during cytotoxic lymphocyte differentiation. To gain insights into the transcriptional regulation of *UNC13D*, the gene encoding Munc13-4, we utilized a previously identified patient mutation in a conserved region in intron 1 that abrogated mRNA transcription and protein expression. This mutation abolished binding of the transcription factor ELF1 as well as recruitment of STAT4 and the chromatin remodeler BRG1 to intron 1. This resulted in decreased transcription of the conventional and complete loss of an alternative transcript, which is most likely indispensable for lytic granule release. Similar to Munc13-4, STAT4 and BRG1 were upregulated during NK cell and $CD8^+$ T cell differentiation and induced upon TCR stimulation of naïve $CD8^+$ T cells *in vitro*. Knockdown of STAT4 prevented the induction of Munc13-4 expression. We thus uncovered a central role for STAT4-mediated chromatin remodeling and DNA accessibility in the transcriptional regulation of lymphocyte cytotoxicity. As STAT4 has also been shown to be critical for IFN-γ production, manipulation of STAT4 signaling could therefore constitute a promising avenue to treat immunopathology associated with cytotoxic lymphocyte function. The role of the newly discovered alternative Munc13-4 isoform in lymphocyte cytotoxicity is subject of ongoing investigations in the lab and will shed new light on the events governing lytic granule exocytosis.

CMV infection leaves a pronounced imprint on the human immune system in general and the NK cell repertoire in particular as specific NK cell subsets expand and persist in CMV^+ individuals. Our investigations in **paper II** revealed that such CMV-associated, adaptive NK cells lose expression of the intracellular signaling molecules FcεRγ, SYK and EAT-2, thereby altering signaling properties downstream of key activating NK cell receptors and target cell recognition. Such silencing occurred in a seemingly probabilistic manner and correlated with DNA hypermethylation at promoter regions. Genome-wide DNA methylation patterns of adaptive NK cells paralleled those of differentiated effector $CD8^+$ T cells and were markedly distinct from canonical NK cells. Adaptive NK cell differentiation was furthermore accompanied by downregulation of the transcription factor PLZF, rendering cell unresponsive to the innate cytokines IL-12 and IL-18. Importantly, adaptive NK cells excelled at ADCC but failed to respond to activated, autologous T cells, implicating a specialized role to eliminate virus infected cells while potentially allowing for enhanced adaptive immune responses. Moreover, utilizing samples from patients with bone marrow disorders, our results in **papers III** and **IV** uncovered that adaptive NK cells persist long-term in the absence of HSPCs. These findings thus extend our knowledge concerning the mechanisms influencing NK cell specialization and survival. This could ultimately advance protocols for the expansion of specific, long-lived and highly functional NK cell subsets for the targeted immunotherapy of cancers.

Tissue-resident $CD8^+$ T (T_{RM}) cells mount rapid immune responses against invading pathogens at barrier sites such as the skin, gut mucosa and the female reproductive tract. While providing protection, they can also contribute to local immunopathology as evidenced by the focal skin diseases vitiligo and psoriasis. In **paper V** we found that healthy human skin harbors $CD8^+$ T_{RM} subsets with distinct effector functions, which can be distinguished by expression of the marker CD49a. Freshly isolated epidermal $CD103^+CD49a^+$ cells were poised for cytotoxicity and rapidly induced perforin and granzyme B expression upon IL-15 priming, facilitating target cell killing. Furthermore, activated epidermal $CD103^+CD49a^+$ cells produced INF-γ, while epidermal $CD103^+CD49a^-$ cells preferentially released IL-17. This functional dichotomy of $CD8^+$ T_{RM} cells was preserved in other tissues such as gut and cervical mucosa as well as lesional skin from vitiligo and psoriasis patients. Importantly, vitiligo lesions contained dramatically increased frequencies of epidermal and dermal $CD103^+CD49a^+$ $CD8^+$ T_{RM} cells. These cells readily expressed perforin and granzyme B and were potent producers of IFN-γ, thereby most likely contributing to melanocyte destruction associated with vitiligo. In contrast, $CD49a^-$ T_{RM} cells producing IL-17 were enriched in the epidermis of psoriasis lesions, driving local hyperinflammation. We thus uncovered a functional specialization of $CD8^+$ T_{RM} subsets in human skin and how those subsets may contribute to disease. Given that IL-15 stimulation primed cells for cytotoxicity and significantly increased production of both IFN-γ and IL-17, IL-15 signaling may be exploited to broadly alleviate $CD8^+$ T_{RM}-associated autoimmune conditions or to mobilize cells for cancer immunotherapy.

4 ACKNOWLEDGEMENTS

Throughout the years, I was incredibly fortunate to meet and interact with many amazing and talented people that contributed immensely to my personal and scientific development.

First and foremost, I have to express my deepest gratitude to my main supervisor **Yenan Bryceson**, who took me on as a Master's and later PhD student and without whom I would not be where I am today. You have been a true role model with an incredible passion for scientific discovery and I will always be inspired by your work ethic and perseverance as well as your attitude towards life.

Thanks to my co-supervisor **Hans-Gustaf Ljunggren**, who gave me the opportunity to join CIM and whose experience and insights provided invaluable guidance and support during my PhD studies.

Importantly, thanks to all co-authors and collaborators, especially:

Frank Cichocki in the lab of **Jeff Miller** for all the contributions to so many projects during your time in Stockholm and back in Minnesota. Looking forward to many great collaborative projects in the future!

Cindy Dunbar for welcoming me into your lab and making those *GATA2* and PNH stories possible. The rest of the Dunbar lab for taking good care of me while visiting, in particular **Tom**, **Moonjung**, **Joy** and **Marcus.**

Liv Eidsmo and **Stanley** for anything tissue related and impressive work on the CD49a paper. Looking forward to fruitful collaborations to come.

Thanks to all past and present members as well as associates of the YTB group:

Sam for teaching me everything flow and NK cell related when I first arrived in the lab, providing me with hot soup when sick and an endless supply of lab stories to tell future generations. **Jakob** for efficiency, multitasking, positivity and having life figured out; you are truly inspirational. **Steph** for always keeping a cool head and trying to get me started on the confocal; I promise I'll pick it up again at some point. **Tim** for tremendous help with sorting, sequencing and hosting me in Bergen; I owe you big time. **Hongya** for badminton, hotpot, lunches and lifts; looking forward to working on some joint projects again. **Matthias** for accuracy, knowing everything about cloning and getting my luggage back almost in time. **Bianca** for amazingly fast and competent help with molecular biology and bioinformatics; I will always be impressed by your skills. **Lamberto** for ham, cheese and taking over the PTPN22 project; I'm glad you stuck around after your Master's. **Jelve** for constant support and running the lab; you are the best. **Martha** for your enthusiasm regarding granule trafficking and exocytosis; your perseverance will pay off. **Giovanna, Beatrice, Donatella** and **Irene** for injecting some Southern European spirit and sociality into the group. **Tak** for Japanese chocolates and translations. **Saeed** for new barbecue experiences. **Ram** for bioinformatics (finally). **Sigrun** for helping out and adapting so readily. **Marie** for cookies and genetics. **Tamara, Angel** and **Misty** for good spirits.

Vivien (and **Julie),** thanks for great years in Flemingsberg and Stockholm as well as showing us around in Paris and Southern France. Looking forward to many more visits wherever we might end up.

Thanks to all past and present **CIM**ers for great times in and outside the lab:

Aline P., **Aline v.A.**, **Anna**, **Benedikt**, **Caroline**, **Christine**, **David** for spontaneous dinners and Eurovision BBQs, **Dominic**, **Ebba**, **Edwin**, **Egle**, **Erika, Erna** (let's get you snowboarding again soon)**, Ginny, Jagadesh, JB, Joana, Julia H., Julia U., Julius and Lydia, Kim, Lisa, Luca, Magda, Marianne, Martin I.** for scientific enthusiasm**, Michal, Monica E., Monika B., Nicole, Nikolai** for realism and canoeing**, Oscar** for root canals and stitches**, Pär** for good times in the snow**, Puran** for cocktails, dinners, brunches and private concerts**, Renata, Robban, Sandra, Sanna** for hosting us in Tokyo**, Sebastian, Senait, Shrikant, Sofia, Su, Themis, Vicky, Jakob Michaelsson** for anything T cell related at any time, **Kalle Malmberg** for endless enthusiasm and great scientific input, **Niklas Björkström** for fishing tips and **Jenny Mjösberg** for letting us trash your kitchen anytime we are having group dinners (it's only getting worse).

The late CIM leadership team **Anna Norrby-Teglund, Johan Sandberg** and **Malin Flodström-Tullberg**.

The past and present CIM technical and administrative team **Margit, Lena, Elisabeth, Annette** and **Carina.**

Thanks to all members of **HERM** for welcoming us to a fresh, stimulating environment:

Aditya, Allegra, Arnika, Ayla, Caroline, Charlotte, Desmond, Edda, Fawaz, Filip, Gözde, Hani, Huthayfa, Lakshmi, Lucia, Makoto, Marios, Michael, Monika, Nadir, Pingnan, Shabnam, Sridharan, Stephan, Stina, Teresa, Thibault, Thuy, Yaser, Ying as well as **Evren Alici, Hong Qian, Julian Walfridsson, Matthias Carlsten, Sten Eirik Jacobsen, Robert Månsson, Petter Woll, Petter Höglund** and anyone I forgot. You made the transition easy and enjoyable and I am grateful to be a part of HERM. Special thanks to **Iyadh** for heroic efforts to maintain a state-of-the-art flow facility and to keep things running smoothly.

Thanks to **Eva Hellström Lindberg** for passionately and efficiently leading HERM, as well as the technical and administrative team around **Monica, Annette, Lili,** and **Sri** for keeping everything afloat.

Thanks to **the Svedin family** for providing a second home in Stockholm and **my family** for endless support and encouragement throughout the years.

Emma, thank you for never getting tired of me and my quirks as well as for sparking my interest in travelling the world together. There is so much more to see and do!

5 REFERENCES

[1] Parkin J, Cohen B. An overview of the immune system. Lancet (London, England) 2001;357:1777–89. doi:10.1016/S0140-6736(00)04904-7.

[2] de Saint Basile G, Ménasché G, Fischer A. Molecular mechanisms of biogenesis and exocytosis of cytotoxic granules. Nat Rev Immunol 2010;10:568–79. doi:10.1038/nri2803.

[3] Caligiuri MA. Human natural killer cells. Blood 2008;112.

[4] Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon- : an overview of signals, mechanisms and functions. J Leukoc Biol 2003;75:163–89. doi:10.1189/jlb.0603252.

[5] Sedger LM, McDermott MF. TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants - past, present and future. Cytokine Growth Factor Rev 2014;25:453–72. doi:10.1016/j.cytogfr.2014.07.016.

[6] Maurer M, von Stebut E. Macrophage inflammatory protein-1. Int J Biochem Cell Biol 2004;36:1882–6. doi:10.1016/j.biocel.2003.10.019.

[7] Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells — a proposal for uniform nomenclature. Nat Rev Immunol 2013;13:145–9. doi:10.1038/nri3365.

[8] Artis D, Spits H. The biology of innate lymphoid cells. Nature 2015;517:293–301. doi:10.1038/nature14189.

[9] Kiessling R, Klein E, Pross H, Wigzell H. „Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol 1975;5:117–21. doi:10.1002/eji.1830050209.

[10] Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. Int J Cancer 1975;16:230–9. doi:10.1002/ijc.2910160205.

[11] Kiessling R, Hochman PS, Haller O, Shearer GM, Wigzell H, Cudkowicz G. Evidence for a similar or common mechanism for natural killer cell activity and resistance to hemopoietic grafts. Eur J Immunol 1977;7:655–63. doi:10.1002/eji.1830070915.

[12] Kärre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H–2-deficient lymphoma variants suggests alternative immune defence strategy. Nature 1986;319:675–8. doi:10.1038/319675a0.

[13] Orr MT, Lanier LL. Inhibitory Ly49 Receptors on Mouse Natural Killer Cells. Curr. Top. Microbiol. Immunol., vol. 350, 2010, p. 67–87. doi:10.1007/82_2010_85.

[14] Moretta L, Moretta A. Killer immunoglobulin-like receptors. Curr Opin Immunol 2004;16:626–33. doi:10.1016/j.coi.2004.07.010.

[15] Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song Y-J, Yang L, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 2005;436:709–13. doi:10.1038/nature03847.

[16] Anfossi N, André P, Guia S, Falk CS, Roetynck S, Stewart CA, et al. Human NK cell education by inhibitory receptors for MHC class I. Immunity 2006;25:331–42. doi:10.1016/j.immuni.2006.06.013.

[17] Brodin P, Lakshmikanth T, Johansson S, Karre K, Hoglund P. The strength of inhibitory input during education quantitatively tunes the functional responsiveness of individual natural killer cells. Blood 2009;113:2434–41. doi:10.1182/blood-2008-05-156836.

[18] Höglund P, Brodin P. Current perspectives of natural killer cell education by MHC class I molecules. Nat Rev Immunol 2010;10:724–34. doi:10.1038/nri2835.

[19] Narni-Mancinelli E, Ugolini S, Vivier E. Tuning the threshold of natural killer cell responses. Curr Opin Immunol 2013;25:53–8. doi:10.1016/j.coi.2012.11.005.

[20] Goodridge JP, Önfelt B, Malmberg K-J. Newtonian cell interactions shape natural killer cell education. Immunol Rev 2015;267:197–213. doi:10.1111/imr.12325.

[21] Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol 2001;22:633–40. doi:10.1016/S1471-4906(01)02060-9.

[22] Sepulveda FE, Maschalidi S, Vosshenrich CAJ, Garrigue A, Kurowska M, Menasche G, et al. A novel immunoregulatory role for NK-cell cytotoxicity in protection from HLH-like immunopathology in mice. Blood 2015;125:1427–34. doi:10.1182/blood-2014-09-602946.

[23] Meeths M, Chiang SCC, Löfstedt A, Müller M-L, Tesi B, Henter J-I, et al. Pathophysiology and spectrum of diseases caused by defects in lymphocyte cytotoxicity. Exp Cell Res 2014;325:10–7. doi:10.1016/j.yexcr.2014.03.014.

[24] Geller MA, Miller JS. Use of allogeneic NK cells for cancer immunotherapy. Immunotherapy 2011;3:1445–59. doi:10.2217/imt.11.131.

[25] Raulet DH, Vance RE, McMahon CW. R EGULATION OF THE N ATURAL K ILLER C ELL R ECEPTOR R EPERTOIRE. Annu Rev Immunol 2001;19:291–330. doi:10.1146/annurev.immunol.19.1.291.

[26] Bryceson YT, March ME, Ljunggren H-G, Long EO. Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion. Blood 2006;107:159–66. doi:10.1182/blood-2005-04-1351.

[27] Kim HS, Das A, Gross CC, Bryceson YT, Long EO. Synergistic signals for natural cytotoxicity are required to overcome inhibition by c-Cbl ubiquitin ligase. Immunity 2010;32:175–86. doi:10.1016/j.immuni.2010.02.004.

[28] Kim HS, Long EO. Complementary Phosphorylation Sites in the Adaptor Protein SLP-76 Promote Synergistic Activation of Natural Killer Cells. Sci Signal 2012;5:ra49-ra49. doi:10.1126/scisignal.2002754.

[29] Bryceson YT, March ME, Barber DF, Ljunggren H-G, Long EO. Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. J Exp Med 2005;202:1001–12. doi:10.1084/jem.20051143.

[30] Hsu H-T, Mace EM, Carisey AF, Viswanath DI, Christakou AE, Wiklund M, et al. NK cells converge lytic granules to promote cytotoxicity and prevent bystander killing. J Cell Biol 2016;215:jcb.201604136. doi:10.1083/jcb.201604136.

[31] Long EO. Negative signaling by inhibitory receptors: the NK cell paradigm. Immunol Rev 2008;224:70–84. doi:10.1111/j.1600-065X.2008.00660.x.

[32] Guia S, Jaeger BN, Piatek S, Mailfert S, Trombik T, Fenis A, et al. Confinement of Activating Receptors at the Plasma Membrane Controls Natural Killer Cell Tolerance. Sci Signal 2011;4:ra21-ra21. doi:10.1126/scisignal.2001608.

[33] Marçais A, Marotel M, Degouve S, Koenig A, Fauteux-Daniel S, Drouillard A, et al. High mTOR activity is a hallmark of reactive natural killer cells and amplifies early signaling through activating receptors. Elife 2017;6. doi:10.7554/eLife.26423.

[34] Brooks AG, Posch PE, Scorzelli CJ, Borrego F, Coligan JE. NKG2A complexed with CD94 defines a novel inhibitory natural killer cell receptor. J Exp Med 1997;185:795–800.

[35] Braud VM, Allan DS, O'Callaghan CA, Söderström K, D'Andrea A, Ogg GS, et al. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature 1998;391:795–9. doi:10.1038/35869.

[36] Lee N, Llano M, Carretero M, Ishitani A, Navarro F, López-Botet M, et al. HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. Proc Natl Acad Sci U S A 1998;95:5199–204.

[37] Braud V, Yvonne Jones E, McMichael A. The human major histocompatibility complex class Ib molecule HLA-E binds signal sequence-derived peptides with primary anchor residues at positions 2 and 9. Eur J Immunol 1997;27:1164–9. doi:10.1002/eji.1830270517.

[38] Olcese L, Lang P, Vély F, Cambiaggi A, Marguet D, Bléry M, et al. Human and mouse killer-cell inhibitory receptors recruit PTP1C and PTP1D protein tyrosine phosphatases.

J Immunol 1996;156:4531–4.

[39] Stebbins CC, Watzl C, Billadeau DD, Leibson PJ, Burshtyn DN, Long EO. Vav1 dephosphorylation by the tyrosine phosphatase SHP-1 as a mechanism for inhibition of cellular cytotoxicity. Mol Cell Biol 2003;23:6291–9.

[40] Burshtyn DN, Shin J, Stebbins C, Long EO. Adhesion to target cells is disrupted by the killer cell inhibitory receptor. Curr Biol 2000;10:777–80.

[41] Abeyweera TP, Kaissar M, Huse M. Inhibitory Receptor Signaling Destabilizes Immunological Synapse Formation in Primary NK Cells. Front Immunol 2013;4:410. doi:10.3389/fimmu.2013.00410.

[42] Huse M, Catherine Milanoski S, Abeyweera TP. Building tolerance by dismantling synapses: inhibitory receptor signaling in natural killer cells. Immunol Rev 2013;251:143–53. doi:10.1111/imr.12014.

[43] Peterson ME, Long EO. Inhibitory Receptor Signaling via Tyrosine Phosphorylation of the Adaptor Crk. Immunity 2008;29:578–88. doi:10.1016/j.immuni.2008.07.014.

[44] Lanier LL, Corliss B, Wu J, Phillips JH. Association of DAP12 with activating CD94/NKG2C NK cell receptors. Immunity 1998;8:693–701.

[45] Moretta L, Moretta A. Unravelling natural killer cell function: triggering and inhibitory human NK receptors. EMBO J 2004;23:255–9. doi:10.1038/sj.emboj.7600019.

[46] Lanier LL. NK CELL RECOGNITION. Annu Rev Immunol 2005;23:225–74. doi:10.1146/annurev.immunol.23.021704.115526.

[47] Lanier LL, Kipps TJ, Phillips JH. Functional properties of a unique subset of cytotoxic CD3+ T lymphocytes that express Fc receptors for IgG (CD16/Leu-11 antigen). J Exp Med 1985;162:2089–106.

[48] Kruse PH, Matta J, Ugolini S, Vivier E. Natural cytotoxicity receptors and their ligands. Immunol Cell Biol 2014;92:221–9. doi:10.1038/icb.2013.98.

[49] Brandt CS, Baratin M, Yi EC, Kennedy J, Gao Z, Fox B, et al. The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans. J Exp Med 2009;206:1495–503. doi:10.1084/jem.20090681.

[50] Matta J, Baratin M, Chiche L, Forel J-M, Cognet C, Thomas G, et al. Induction of B7-H6, a ligand for the natural killer cell-activating receptor NKp30, in inflammatory conditions. Blood 2013;122:394–404. doi:10.1182/blood-2013-01-481705.

[51] Tomasec P, Braud VM, Rickards C, Powell MB, McSharry BP, Gadola S, et al. Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. Science 2000;287:1031.

[52] Nattermann J, Nischalke HD, Hofmeister V, Kupfer B, Ahlenstiel G, Feldmann G, et al. HIV-1 infection leads to increased HLA-E expression resulting in impaired function of natural killer cells. Antivir Ther 2005;10:95–107.

[53] Nattermann J, Nischalke HD, Hofmeister V, Ahlenstiel G, Zimmermann H, Leifeld L, et al. The HLA-A2 restricted T cell epitope HCV core 35-44 stabilizes HLA-E expression and inhibits cytolysis mediated by natural killer cells. Am J Pathol 2005;166:443–53. doi:10.1016/S0002-9440(10)62267-5.

[54] Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. Cell 1994;76:263–74.

[55] Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 2008;9:495–502. doi:10.1038/ni1581.

[56] Guerra N, Tan YX, Joncker NT, Choy A, Gallardo F, Xiong N, et al. NKG2D-Deficient Mice Are Defective in Tumor Surveillance in Models of Spontaneous Malignancy. Immunity 2008;28:571–80. doi:10.1016/j.immuni.2008.02.016.

[57] Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. Nat Rev Immunol 2003;3:781–90. doi:10.1038/nri1199.

[58] Wu J, Song Y, Bakker AB, Bauer S, Spies T, Lanier LL, et al. An activating

immunoreceptor complex formed by NKG2D and DAP10. Science 1999;285:730–2.

[59] Gilfillan S, Ho EL, Cella M, Yokoyama WM, Colonna M. NKG2D recruits two distinct adapters to trigger NK cell activation and costimulation. Nat Immunol 2002;3:1150–5. doi:10.1038/ni857.

[60] Billadeau DD, Upshaw JL, Schoon RA, Dick CJ, Leibson PJ. NKG2D-DAP10 triggers human NK cell–mediated killing via a Syk-independent regulatory pathway. Nat Immunol 2003;4:557–64. doi:10.1038/ni929.

[61] Graham DB, Cella M, Giurisato E, Fujikawa K, Miletic A V, Kloeppel T, et al. Vav1 controls DAP10-mediated natural cytotoxicity by regulating actin and microtubule dynamics. J Immunol 2006;177:2349–55.

[62] Upshaw JL, Arneson LN, Schoon RA, Dick CJ, Billadeau DD, Leibson PJ. NKG2D-mediated signaling requires a DAP10-bound Grb2-Vav1 intermediate and phosphatidylinositol-3-kinase in human natural killer cells. Nat Immunol 2006;7:524–32. doi:10.1038/ni1325.

[63] Cannons JL, Tangye SG, Schwartzberg PL. SLAM Family Receptors and SAP Adaptors in Immunity. Annu Rev Immunol 2011;29:665–705. doi:10.1146/annurev-immunol-030409-101302.

[64] Latour S, Roncagalli R, Chen R, Bakinowski M, Shi X, Schwartzberg PL, et al. Binding of SAP SH2 domain to FynT SH3 domain reveals a novel mechanism of receptor signalling in immune regulation. Nat Cell Biol 2003;5:149–54. doi:10.1038/ncb919.

[65] Pérez-Quintero L-A, Roncagalli R, Guo H, Latour S, Davidson D, Veillette A. EAT-2, a SAP-like adaptor, controls NK cell activation through phospholipase Cγ, Ca^{++}, and Erk, leading to granule polarization. J Exp Med 2014;211:727–42. doi:10.1084/jem.20132038.

[66] Tassi I, Colonna M. The cytotoxicity receptor CRACC (CS-1) recruits EAT-2 and activates the PI3K and phospholipase Cgamma signaling pathways in human NK cells. J Immunol 2005;175:7996–8002.

[67] Eissmann P, Beauchamp L, Wooters J, Tilton JC, Long EO, Watzl C. Molecular basis for positive and negative signaling by the natural killer cell receptor 2B4 (CD244). Blood 2005;105:4722–9. doi:10.1182/blood-2004-09-3796.

[68] Engel P, Eck MJ, Terhorst C. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. Nat Rev Immunol 2003;3:813–21. doi:10.1038/nri1202.

[69] Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. Immunol Rev 2005;203:180–99. doi:10.1111/j.0105-2896.2005.00230.x.

[70] Wu N, Zhong M-C, Roncagalli R, Pérez-Quintero L-A, Guo H, Zhang Z, et al. A hematopoietic cell–driven mechanism involving SLAMF6 receptor, SAP adaptors and SHP-1 phosphatase regulates NK cell education. Nat Immunol 2016;17:387–96. doi:10.1038/ni.3369.

[71] Meazza R, Falco M, Marcenaro S, Loiacono F, Canevali P, Bellora F, et al. Inhibitory 2B4 contributes to NK cell education and immunological derangements in XLP1 patients. Eur J Immunol 2017;47:1051–61. doi:10.1002/eji.201646885.

[72] Iguchi-Manaka A, Kai H, Yamashita Y, Shibata K, Tahara-Hanaoka S, Honda S, et al. Accelerated tumor growth in mice deficient in DNAM-1 receptor. J Exp Med 2008;205:2959–64. doi:10.1084/jem.20081611.

[73] Castriconi R, Dondero A, Corrias MV, Lanino E, Pende D, Moretta L, et al. Natural Killer Cell-Mediated Killing of Freshly Isolated Neuroblastoma Cells. Cancer Res 2004;64:9180–4. doi:10.1158/0008-5472.CAN-04-2682.

[74] Carlsten M, Bjorkstrom NK, Norell H, Bryceson Y, van Hall T, Baumann BC, et al. DNAX Accessory Molecule-1 Mediated Recognition of Freshly Isolated Ovarian Carcinoma by Resting Natural Killer Cells. Cancer Res 2007;67:1317–25. doi:10.1158/0008-

5472.CAN-06-2264.

[75] Lakshmikanth T, Burke S, Ali TH, Kimpfler S, Ursini F, Ruggeri L, et al. NCRs and DNAM-1 mediate NK cell recognition and lysis of human and mouse melanoma cell lines in vitro and in vivo. J Clin Invest 2009;119:1251–63. doi:10.1172/JCI36022.

[76] Bottino C, Castriconi R, Pende D, Rivera P, Nanni M, Carnemolla B, et al. Identification of PVR (CD155) and Nectin-2 (CD112) as Cell Surface Ligands for the Human DNAM-1 (CD226) Activating Molecule. J Exp Med 2003;198:557–67. doi:10.1084/jem.20030788.

[77] Pende D, Castriconi R, Romagnani P, Spaggiari GM, Marcenaro S, Dondero A, et al. Expression of the DNAM-1 ligands, Nectin-2 (CD112) and poliovirus receptor (CD155), on dendritic cells: relevance for natural killer-dendritic cell interaction. Blood 2006;107:2030–6. doi:10.1182/blood-2005-07-2696.

[78] Ardolino M, Zingoni A, Cerboni C, Cecere F, Soriani A, Iannitto ML, et al. DNAM-1 ligand expression on Ag-stimulated T lymphocytes is mediated by ROS-dependent activation of DNA-damage response: relevance for NK-T cell interaction. Blood 2011;117:4778–86. doi:10.1182/blood-2010-08-300954.

[79] Shibuya K, Lanier LL, Phillips JH, Ochs HD, Shimizu K, Nakayama E, et al. Physical and functional association of LFA-1 with DNAM-1 adhesion molecule. Immunity 1999;11:615–23.

[80] Enqvist M, Ask EH, Forslund E, Carlsten M, Abrahamsen G, Béziat V, et al. Coordinated expression of DNAM-1 and LFA-1 in educated NK cells. J Immunol 2015;194:4518–27. doi:10.4049/jimmunol.1401972.

[81] Zhang Z, Wu N, Lu Y, Davidson D, Colonna M, Veillette A. DNAM-1 controls NK cell activation via an ITT-like motif. J Exp Med 2015;212:2165–82. doi:10.1084/jem.20150792.

[82] Upshaw JL, Schoon RA, Dick CJ, Billadeau DD, Leibson PJ. The isoforms of phospholipase C-gamma are differentially used by distinct human NK activating receptors. J Immunol 2005;175:213–8.

[83] Tassi I, Presti R, Kim S, Yokoyama WM, Gilfillan S, Colonna M. Phospholipase C-gamma 2 is a critical signaling mediator for murine NK cell activating receptors. J Immunol 2005;175:749–54.

[84] Ombrello MJ, Remmers EF, Sun G, Freeman AF, Datta S, Torabi-Parizi P, et al. Cold Urticaria, Immunodeficiency, and Autoimmunity Related to *PLCG2* Deletions. N Engl J Med 2012;366:330–8. doi:10.1056/NEJMoa1102140.

[85] Hesslein DGT, Palacios EH, Sun JC, Beilke JN, Watson SR, Weiss A, et al. Differential requirements for CD45 in NK-cell function reveal distinct roles for Syk-family kinases. Blood 2011;117:3087–95. doi:10.1182/blood-2010-06-292219.

[86] Roncagalli R, Taylor JER, Zhang S, Shi X, Chen R, Cruz-Munoz M-E, et al. Negative regulation of natural killer cell function by EAT-2, a SAP-related adaptor. Nat Immunol 2005;6:1002–10. doi:10.1038/ni1242.

[87] Dong Z, Cruz-Munoz M-E, Zhong M-C, Chen R, Latour S, Veillette A. Essential function for SAP family adaptors in the surveillance of hematopoietic cells by natural killer cells. Nat Immunol 2009;10:973–80. doi:10.1038/ni.1763.

[88] Haller O, Kiessling R, Orn A, Wigzell H. Generation of natural killer cells: an autonomous function of the bone marrow. J Exp Med 1977;145.

[89] Miller JS, Verfaillie C, McGlave P. The generation of human natural killer cells from CD34+/DR- primitive progenitors in long-term bone marrow culture. Blood 1992;80:2182–7.

[90] Miller JS, Alley KA, McGlave P. Differentiation of natural killer (NK) cells from human primitive marrow progenitors in a stroma-based long-term culture system: identification of a CD34+7+ NK progenitor. Blood 1994;83:2594–601.

[91] Sánchez MJ, Muench MO, Roncarolo MG, Lanier LL, Phillips JH. Identification of a common T/natural killer cell progenitor in human fetal thymus. J Exp Med

1994;180:569–76.

[92] Jaleco AC, Blom B, Res P, Weijer K, Lanier LL, Phillips JH, et al. Fetal liver contains committed NK progenitors, but is not a site for development of CD34+ cells into T cells. J Immunol 1997;159.

[93] Freud AG, Becknell B, Roychowdhury S, Mao HC, Ferketich AK, Nuovo GJ, et al. A human CD34(+) subset resides in lymph nodes and differentiates into CD56bright natural killer cells. Immunity 2005;22:295–304. doi:10.1016/j.immuni.2005.01.013.

[94] Freud AG, Yokohama A, Becknell B, Lee MT, Mao HC, Ferketich AK, et al. Evidence for discrete stages of human natural killer cell differentiation in vivo. J Exp Med 2006;203:1033–43. doi:10.1084/jem.20052507.

[95] Freud AG, Caligiuri MA. Human natural killer cell development. Immunol Rev 2006;214:56–72. doi:10.1111/j.1600-065X.2006.00451.x.

[96] Mrózek E, Anderson P, Caligiuri MA. Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. Blood 1996;87:2632–40.

[97] Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, et al. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell 1993;73:147–57.

[98] Gilmour KC, Fujii H, Cranston T, Davies EG, Kinnon C, Gaspar HB. Defective expression of the interleukin-2/interleukin-15 receptor beta subunit leads to a natural killer cell-deficient form of severe combined immunodeficiency. Blood 2001;98:877–9.

[99] Macchi P, Villa A, Giliani S, Sacco MG, Frattini A, Porta F, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature 1995;377:65–8. doi:10.1038/377065a0.

[100] Huntington ND, Legrand N, Alves NL, Jaron B, Weijer K, Plet A, et al. IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. J Exp Med 2009;206:25–34. doi:10.1084/jem.20082013.

[101] Scoville SD, Freud AG, Caligiuri MA. Modeling Human Natural Killer Cell Development in the Era of Innate Lymphoid Cells. Front Immunol 2017;8:360. doi:10.3389/fimmu.2017.00360.

[102] Renoux VM, Zriwil A, Peitzsch C, Michaëlsson J, Friberg D, Soneji S, et al. Identification of a Human Natural Killer Cell Lineage-Restricted Progenitor in Fetal and Adult Tissues. Immunity 2015;43:394–407. doi:10.1016/j.immuni.2015.07.011.

[103] Freud AG, Keller KA, Scoville SD, Mundy-Bosse BL, Cheng S, Youssef Y, et al. NKp80 Defines a Critical Step during Human Natural Killer Cell Development. Cell Rep 2016;16:379–91. doi:10.1016/j.celrep.2016.05.095.

[104] Gordon SM, Chaix J, Rupp LJ, Wu J, Madera S, Sun JC, et al. The Transcription Factors T-bet and Eomes Control Key Checkpoints of Natural Killer Cell Maturation. Immunity 2012;36:55–67. doi:10.1016/j.immuni.2011.11.016.

[105] Intlekofer AM, Takemoto N, Wherry EJ, Longworth SA, Northrup JT, Palanivel VR, et al. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. Nat Immunol 2005;6:1236–44. doi:10.1038/ni1268.

[106] Lanier LL, Testi R, Bindl J, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. J Exp Med 1989;169.

[107] Nagler A, Lanier LL, Cwirla S, Phillips JH. Comparative studies of human FcRIII-positive and negative natural killer cells. J Immunol 1989;143.

[108] Ferlazzo G, Thomas D, Lin S-L, Goodman K, Morandi B, Muller WA, et al. The Abundant NK Cells in Human Secondary Lymphoid Tissues Require Activation to Express Killer Cell Ig-Like Receptors and Become Cytolytic. J Immunol 2004;172.

[109] Chan A, Hong D-L, Atzberger A, Kollnberger S, Filer AD, Buckley CD, et al. CD56bright human NK cells differentiate into CD56dim cells: role of contact with peripheral

fibroblasts. J Immunol 2007;179:89–94.

[110] Romagnani C, Juelke K, Falco M, Morandi B, D'Agostino A, Costa R, et al. CD56brightCD16- killer Ig-like receptor- NK cells display longer telomeres and acquire features of CD56dim NK cells upon activation. J Immunol (Baltimore, Md 1950) 2007;178:4947–55.

[111] OUYANG Q, BAERLOCHER G, VULTO I, LANSDORP PM. Telomere Length in Human Natural Killer Cell Subsets. Ann N Y Acad Sci 2007;1106:240–52. doi:10.1196/annals.1392.001.

[112] Jacobs R, Stoll M, Stratmann G, Leo R, Link H, Schmidt RE. CD16- CD56+ natural killer cells after bone marrow transplantation. Blood 1992;79:3239–44.

[113] Dulphy N, Haas P, Busson M, Belhadj S, Peffault de Latour R, Robin M, et al. An unusual CD56(bright) CD16(low) NK cell subset dominates the early posttransplant period following HLA-matched hematopoietic stem cell transplantation. J Immunol 2008;181:2227–37.

[114] Béziat V, Descours B, Parizot C, Debré P, Vieillard V. NK cell terminal differentiation: correlated stepwise decrease of NKG2A and acquisition of KIRs. PLoS One 2010;5:e11966. doi:10.1371/journal.pone.0011966.

[115] Campbell JJ, Qin S, Unutmaz D, Soler D, Murphy KE, Hodge MR, et al. Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. J Immunol 2001;166:6477–82.

[116] Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. Blood 2001;97.

[117] Jacobs R, Hintzen G, Kemper A, Beul K, Kempf S, Behrens G, et al. CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. Eur J Immunol 2001;31:3121–7. doi:10.1002/1521-4141(2001010)31:10<3121::AID-IMMU3121>3.0.CO;2-4.

[118] Juelke K, Killig M, Luetke-Eversloh M, Parente E, Gruen J, Morandi B, et al. CD62L expression identifies a unique subset of polyfunctional CD56dim NK cells. Blood 2010;116:1299–307. doi:10.1182/blood-2009-11-253286.

[119] Björkström NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA, et al. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. Blood 2010;116:3853–64. doi:10.1182/blood-2010-04-281675.

[120] Lopez-Vergès S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, et al. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. Blood 2010;116:3865–74. doi:10.1182/blood-2010-04-282301.

[121] Marquardt N, Béziat V, Nyström S, Hengst J, Ivarsson MA, Kekäläinen E, et al. Cutting edge: identification and characterization of human intrahepatic CD49a+ NK cells. J Immunol 2015;194:2467–71. doi:10.4049/jimmunol.1402756.

[122] Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol 2002;2:656–63. doi:10.1038/nri886.

[123] Björkström NK, Ljunggren H-G, Michaëlsson J. Emerging insights into natural killer cells in human peripheral tissues. Nat Rev Immunol 2016;16:310–20. doi:10.1038/nri.2016.34.

[124] Odom CI, Gaston DC, Markert JM, Cassady KA. Human herpesviridae methods of natural killer cell evasion. Adv Virol 2012;2012:359869. doi:10.1155/2012/359869.

[125] Miller-Kittrell M, Sparer TE, Virgin Hw, Soderberg-Naucler C, Altschuler Y, Jones T, et al. Feeling manipulated: cytomegalovirus immune manipulation. Virol J 2009;6:4. doi:10.1186/1743-422X-6-4.

[126] Lanier LL. Evolutionary struggles between NK cells and viruses. Nat Rev Immunol

2008;8:259–68. doi:10.1038/nri2276.

[127] Biron CA, Byron KS, Sullivan JL. Severe Herpesvirus Infections in an Adolescent without Natural Killer Cells. N Engl J Med 1989;320:1731–5. doi:10.1056/NEJM198906293202605.

[128] Orange JS. Natural killer cell deficiency. J Allergy Clin Immunol 2013;132:515–25. doi:10.1016/j.jaci.2013.07.020.

[129] Kuijpers TW, Baars PA, Dantin C, van den Burg M, van Lier RAW, Roosnek E. Human NK cells can control CMV infection in the absence of T cells. Blood 2008;112.

[130] Brown MG, Dokun AO, Heusel JW, Smith HR, Beckman DL, Blattenberger EA, et al. Vital Involvement of a Natural Killer Cell Activation Receptor in Resistance to Viral Infection. Science (80-) 2001;292:934–7. doi:10.1126/science.1060042.

[131] Daniels KA, Devora G, Lai WC, O'Donnell CL, Bennett M, Welsh RM. Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. J Exp Med 2001;194:29–44.

[132] Dokun AO, Kim S, Smith HRC, Kang H-SP, Chu DT, Yokoyama WM. Specific and nonspecific NK cell activation during virus infection. Nat Immunol 2001;2:951–6. doi:10.1038/ni714.

[133] Robbins SH, Tessmer MS, Mikayama T, Brossay L. Expansion and contraction of the NK cell compartment in response to murine cytomegalovirus infection. J Immunol 2004;173:259–66.

[134] Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature 2009;457:557–61. doi:10.1038/nature07665.

[135] Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. Science 2002;296:1323–6. doi:10.1126/science.1070884.

[136] Smith HRC, Heusel JW, Mehta IK, Kim S, Dorner BG, Naidenko O V., et al. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. Proc Natl Acad Sci 2002;99:8826–31. doi:10.1073/pnas.092258599.

[137] Gumá M, Angulo A, Vilches C, Gómez-Lozano N, Malats N, López-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood 2004;104:3664–71. doi:10.1182/blood-2004-05-2058.

[138] Gumá M, Cabrera C, Erkizia I, Bofill M, Clotet B, Ruiz L, et al. Human cytomegalovirus infection is associated with increased proportions of NK cells that express the CD94/NKG2C receptor in aviremic HIV-1-positive patients. J Infect Dis 2006;194:38–41. doi:10.1086/504719.

[139] Gumá M, Budt M, Sáez A, Brckalo T, Hengel H, Angulo A, et al. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. Blood 2006;107:3624–31. doi:10.1182/blood-2005-09-3682.

[140] Lopez-Vergès S, Milush JM, Schwartz BS, Pando MJ, Jarjoura J, York VA, et al. Expansion of a unique $CD57^{+}$NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. Proc Natl Acad Sci U S A 2011;108:14725–32. doi:10.1073/pnas.1110900108.

[141] Foley B, Cooley S, Verneris MR, Pitt M, Curtsinger J, Luo X, et al. Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function. Blood 2012;119:2665–74. doi:10.1182/blood-2011-10-386995.

[142] Luetke-Eversloh M, Hammer Q, Durek P, Nordström K, Gasparoni G, Pink M, et al. Human cytomegalovirus drives epigenetic imprinting of the IFNG locus in NKG2Chi natural killer cells. PLoS Pathog 2014;10:e1004441. doi:10.1371/journal.ppat.1004441.

[143] Björkström NK, Lindgren T, Stoltz M, Fauriat C, Braun M, Evander M, et al. Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J Exp Med 2011;208:13–21. doi:10.1084/jem.20100762.

[144] Béziat V, Dalgard O, Asselah T, Halfon P, Bedossa P, Boudifa A, et al. CMV drives clonal expansion of NKG2C+ NK cells expressing self-specific KIRs in chronic hepatitis patients. Eur J Immunol 2012;42:447–57. doi:10.1002/eji.201141826.

[145] Béziat V, Liu LL, Malmberg J-A, Ivarsson MA, Sohlberg E, Björklund AT, et al. NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. Blood 2013;121:2678–88. doi:10.1182/blood-2012-10-459545.

[146] Hwang I, Zhang T, Scott JM, Kim AR, Lee T, Kakarla T, et al. Identification of human NK cells that are deficient for signaling adaptor FcRγ and specialized for antibody-dependent immune functions. Int Immunol 2012;24:793–802. doi:10.1093/intimm/dxs080.

[147] Zhang T, Scott JM, Hwang I, Kim S. Cutting edge: antibody-dependent memory-like NK cells distinguished by FcRγ deficiency. J Immunol (Baltimore, Md 1950) 2013;190:1402–6. doi:10.4049/jimmunol.1203034.

[148] Costa-Garcia M, Vera A, Moraru M, Vilches C, López-Botet M, Muntasell A. Antibody-Mediated Response of NKG2C ^{bright} NK Cells against Human Cytomegalovirus. J Immunol 2015;194:2715–24. doi:10.4049/jimmunol.1402281.

[149] Firth MA, Madera S, Beaulieu AM, Gasteiger G, Castillo EF, Schluns KS, et al. Nfil3-independent lineage maintenance and antiviral response of natural killer cells. J Exp Med 2013;210:2981–90. doi:10.1084/jem.20130417.

[150] Beaulieu AM, Zawislak CL, Nakayama T, Sun JC. The transcription factor Zbtb32 controls the proliferative burst of virus-specific natural killer cells responding to infection. Nat Immunol 2014;15:546–53. doi:10.1038/ni.2876.

[151] Rölle A, Pollmann J, Ewen E-M, Le VTK, Halenius A, Hengel H, et al. IL-12-producing monocytes and HLA-E control HCMV-driven NKG2C+ NK cell expansion. J Clin Invest 2014;124:5305–16. doi:10.1172/JCI77440.

[152] Nabekura T, Kanaya M, Shibuya A, Fu G, Gascoigne NRJ, Lanier LL. Costimulatory molecule DNAM-1 is essential for optimal differentiation of memory natural killer cells during mouse cytomegalovirus infection. Immunity 2014;40:225–34. doi:10.1016/j.immuni.2013.12.011.

[153] Tsai F-Y, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature 1994;371:221–6. doi:10.1038/371221a0.

[154] Tsai FY, Orkin SH. Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. Blood 1997;89:3636–43.

[155] Bigley V, Haniffa M, Doulatov S, Wang X-N, Dickinson R, McGovern N, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. J Exp Med 2011;208:227–34. doi:10.1084/jem.20101459.

[156] Dickinson RE, Griffin H, Bigley V, Reynard LN, Hussain R, Haniffa M, et al. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. Blood 2011;118:2656–8. doi:10.1182/blood-2011-06-360313.

[157] Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. Blood 2011;118:2653–5. doi:10.1182/blood-2011-05-356352.

[158] Vinh DC, Patel SY, Uzel G, Anderson VL, Freeman AF, Olivier KN, et al. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood 2010;115:1519–29. doi:10.1182/blood-2009-03-208629.

[159] Hahn CN, Chong C-E, Carmichael CL, Wilkins EJ, Brautigan PJ, Li X-C, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid

leukemia. Nat Genet 2011;43:1012–7. doi:10.1038/ng.913.

[160] Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet 2011;43:929–31. doi:10.1038/ng.923.

[161] Pasquet M, Bellanné-Chantelot C, Tavitian S, Prade N, Beaupain B, Larochelle O, et al. High frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMac syndrome, myelodysplasia, and acute myeloid leukemia. Blood 2013;121:822–9. doi:10.1182/blood-2012-08-447367.

[162] Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood 2014;123:809–21. doi:10.1182/blood-2013-07-515528.

[163] Mace EM, Hsu AP, Monaco-Shawver L, Makedonas G, Rosen JB, Dropulic L, et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56bright subset. Blood 2013;121:2669–77. doi:10.1182/blood-2012-09-453969.

[164] Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N, et al. The evolution of cellular deficiency in GATA2 mutation. Blood 2014;123:863–74. doi:10.1182/blood-2013-07-517151.

[165] Brodsky RA. Paroxysmal nocturnal hemoglobinuria. Blood 2014;124:2804–11. doi:10.1182/blood-2014-02-522128.

[166] Zhang Y, Wallace DL, de Lara CM, Ghattas H, Asquith B, Worth A, et al. In vivo kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection. Immunology 2007;121:258–65. doi:10.1111/j.1365-2567.2007.02573.x.

[167] Spits H. Development of αβ t cells in the human thymus. Nat Rev Immunol 2002;2:760–72. doi:10.1038/nri913.

[168] Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. Annu Rev Immunol 2009;27:591–619. doi:10.1146/annurev.immunol.021908.132706.

[169] Sallusto F. Heterogeneity of Human CD4(+) T Cells Against Microbes. Annu Rev Immunol 2016;34:317–34. doi:10.1146/annurev-immunol-032414-112056.

[170] Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature 1988;334:395–402. doi:10.1038/334395a0.

[171] Bassing CH, Swat W, Alt FW. The mechanism and regulation of chromosomal V(D)J recombination. Cell 2002;109 Suppl:S45-55.

[172] Starr TK, Jameson SC, Hogquist KA. P OSITIVE AND N EGATIVE S ELECTION OF T C ELLS. Annu Rev Immunol 2003;21:139–76. doi:10.1146/annurev.immunol.21.120601.141107.

[173] Krangel MS. Mechanics of T cell receptor gene rearrangement. Curr Opin Immunol 2009;21:133–9. doi:10.1016/j.coi.2009.03.009.

[174] Anderson G, Lane PJL, Jenkinson EJ. Generating intrathymic microenvironments to establish T-cell tolerance. Nat Rev Immunol 2007;7:954–63. doi:10.1038/nri2187.

[175] Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P. A direct estimate of the human alphabeta T cell receptor diversity. Science 1999;286:958–61.

[176] Sewell AK. Why must T cells be cross-reactive? Nat Rev Immunol 2012;12:669–77. doi:10.1038/nri3279.

[177] Germain RN. T-cell development and the CD4–CD8 lineage decision. Nat Rev Immunol 2002;2:309–22. doi:10.1038/nri798.

[178] Worbs T, Hammerschmidt SI, Förster R. Dendritic cell migration in health and disease. Nat Rev Immunol 2016;17:30–48. doi:10.1038/nri.2016.116.

[179] Curtsinger JM, Schmidt CS, Mondino A, Lins DC, Kedl RM, Jenkins MK, et al. Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells. J Immunol 1999;162:3256–62.

[180] Curtsinger JM, Johnson CM, Mescher MF. CD8 T cell clonal expansion and development of effector function require prolonged exposure to antigen, costimulation, and signal 3 cytokine. J Immunol 2003;171:5165–71.

[181] Curtsinger JM, Valenzuela JO, Agarwal P, Lins D, Mescher MF. Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation. J Immunol 2005;174:4465–9.

[182] Mescher MF, Curtsinger JM, Agarwal P, Casey KA, Gerner M, Hammerbeck CD, et al. Signals required for programming effector and memory development by CD8+ T cells. Immunol Rev 2006;211:81–92. doi:10.1111/j.0105-2896.2006.00382.x.

[183] Curtsinger JM, Lins DC, Mescher MF. Signal 3 determines tolerance versus full activation of naive CD8 T cells: dissociating proliferation and development of effector function. J Exp Med 2003;197:1141–51. doi:10.1084/jem.20021910.

[184] Appleman LJ, Boussiotis VA. T cell anergy and costimulation. Immunol Rev 2003;192:161–80.

[185] Badovinac VP, Harty JT. CD8(+) T-cell homeostasis after infection: setting the “curve”. Microbes Infect 2002;4:441–7.

[186] Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde SR, Klein MR, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. J Exp Med 1997;186:1407–18.

[187] Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999;401:708–12. doi:10.1038/44385.

[188] Opferman JT, Ober BT, Ashton-Rickardt PG. Linear differentiation of cytotoxic effectors into memory T lymphocytes. Science 1999;283:1745–8.

[189] Wherry EJ, Teichgräber V, Becker TC, Masopust D, Kaech SM, Antia R, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. Nat Immunol 2003;4:225–34. doi:10.1038/ni889.

[190] Yamashita N, Nguyen L, Fahey JL, Clement LT. Phenotypic properties and cytotoxic functions of human CD8+ cells expressing the CD57 antigen. Nat Immun 1993;12:79–91.

[191] Le Priol Y, Puthier D, Lécureuil C, Combadière C, Debré P, Nguyen C, et al. High cytotoxic and specific migratory potencies of senescent CD8+ CD57+ cells in HIV-infected and uninfected individuals. J Immunol 2006;177:5145–54.

[192] Chiang SCC, Theorell J, Entesarian M, Meeths M, Mastafa M, Al-Herz W, et al. Comparison of primary human cytotoxic T-cell and natural killer cell responses reveal similar molecular requirements for lytic granule exocytosis but differences in cytokine production. Blood 2013;121:1345–56. doi:10.1182/blood-2012-07-442558.

[193] Takata H, Takiguchi M. Three memory subsets of human CD8+ T cells differently expressing three cytolytic effector molecules. J Immunol 2006;177:4330–40.

[194] Romero P, Zippelius A, Kurth I, Pittet MJ, Touvrey C, Iancu EM, et al. Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. J Immunol 2007;178:4112–9.

[195] Schluns KS, Lefrançois L. Cytokine control of memory T-cell development and survival. Nat Rev Immunol 2003;3:269–79. doi:10.1038/nri1052.

[196] Smith KA. Interleukin-2: inception, impact, and implications. Science 1988;240:1169–76.

[197] Kalia V, Sarkar S, Subramaniam S, Haining WN, Smith KA, Ahmed R. Prolonged Interleukin-2Rα Expression on Virus-Specific CD8+ T Cells Favors Terminal-Effector Differentiation In Vivo. Immunity 2010;32:91–103. doi:10.1016/j.immuni.2009.11.010.

[198] Pipkin ME, Sacks JA, Cruz-Guilloty F, Lichtenheld MG, Bevan MJ, Rao A. Interleukin-2 and Inflammation Induce Distinct Transcriptional Programs that Promote the Differentiation of Effector Cytolytic T Cells. Immunity 2010;32:79–90.

doi:10.1016/j.immuni.2009.11.012.

[199] Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 2000;288:675–8.

[200] Schluns KS, Williams K, Ma A, Zheng XX, Lefrançois L. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. J Immunol 2002;168:4827–31.

[201] Becker TC, Wherry EJ, Boone D, Murali-Krishna K, Antia R, Ma A, et al. Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. J Exp Med 2002;195:1541–8.

[202] Goldrath AW, Sivakumar P V, Glaccum M, Kennedy MK, Bevan MJ, Benoist C, et al. Cytokine requirements for acute and Basal homeostatic proliferation of naive and memory CD8+ T cells. J Exp Med 2002;195:1515–22.

[203] Schluns KS, Kieper WC, Jameson SC, Lefrançois L. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. Nat Immunol 2000;1:426–32. doi:10.1038/80868.

[204] Joshi NS, Cui W, Chandele A, Lee HK, Urso DR, Hagman J, et al. Inflammation Directs Memory Precursor and Short-Lived Effector CD8+ T Cell Fates via the Graded Expression of T-bet Transcription Factor. Immunity 2007;27:281–95. doi:10.1016/j.immuni.2007.07.010.

[205] Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol 2003;4:1191–8. doi:10.1038/ni1009.

[206] Pearce EL, Shen H. Generation of CD8 T cell memory is regulated by IL-12. J Immunol 2007;179:2074–81.

[207] Takemoto N, Intlekofer AM, Northrup JT, Wherry EJ, Reiner SL. Cutting Edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced CD8+ T cell differentiation. J Immunol 2006;177:7515–9.

[208] Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, et al. Control of effector CD8+ T cell function by the transcription factor Eomesodermin. Science 2003;302:1041–3. doi:10.1126/science.1090148.

[209] Kallies A, Xin A, Belz GT, Nutt SL. Blimp-1 Transcription Factor Is Required for the Differentiation of Effector CD8+ T Cells and Memory Responses. Immunity 2009;31:283–95. doi:10.1016/j.immuni.2009.06.021.

[210] Chang JT, Palanivel VR, Kinjyo I, Schambach F, Intlekofer AM, Banerjee A, et al. Asymmetric T Lymphocyte Division in the Initiation of Adaptive Immune Responses. Science (80-) 2007;315:1687–91. doi:10.1126/science.1139393.

[211] Ciocca ML, Barnett BE, Burkhardt JK, Chang JT, Reiner SL. Cutting Edge: Asymmetric Memory T Cell Division in Response to Rechallenge. J Immunol 2012;188:4145–8. doi:10.4049/jimmunol.1200176.

[212] Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic Instruction of Immunity. Cell 2017;169:570–86. doi:10.1016/j.cell.2017.04.004.

[213] Chang C-H, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. Cell 2013;153:1239–51. doi:10.1016/j.cell.2013.05.016.

[214] Buck MD, O'Sullivan D, Klein Geltink RI, Curtis JD, Chang C-H, Sanin DE, et al. Mitochondrial Dynamics Controls T Cell Fate through Metabolic Programming. Cell 2016;166:63–76. doi:10.1016/j.cell.2016.05.035.

[215] O'Sullivan D, van der Windt GJW, Huang SC-C, Curtis JD, Chang C-H, Buck MD, et al. Memory CD8+ T Cells Use Cell-Intrinsic Lipolysis to Support the Metabolic Programming Necessary for Development. Immunity 2014;41:75–88. doi:10.1016/j.immuni.2014.06.005.

[216] Cui G, Staron MM, Gray SM, Ho P-C, Amezquita RA, Wu J, et al. IL-7-Induced Glycerol Transport and TAG Synthesis Promotes Memory CD8+ T Cell Longevity. Cell 2015;161:750–61. doi:10.1016/j.cell.2015.03.021.

[217] Schenkel JM, Masopust D. Tissue-Resident Memory T Cells. Immunity 2014;41:886–97. doi:10.1016/j.immuni.2014.12.007.

[218] Thome JJC, Farber DL. Emerging concepts in tissue-resident T cells: lessons from humans. Trends Immunol 2015;36:428–35. doi:10.1016/j.it.2015.05.003.

[219] Watanabe R, Gehad A, Yang C, Scott LL, Teague JE, Schlapbach C, et al. Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. Sci Transl Med 2015;7:279ra39. doi:10.1126/scitranslmed.3010302.

[220] Masopust D, Choo D, Vezys V, Wherry EJ, Duraiswamy J, Akondy R, et al. Dynamic T cell migration program provides resident memory within intestinal epithelium. J Exp Med 2010;207:553–64. doi:10.1084/jem.20090858.

[221] Purwar R, Campbell J, Murphy G, Richards WG, Clark RA, Kupper TS. Resident Memory T Cells (TRM) Are Abundant in Human Lung: Diversity, Function, and Antigen Specificity. PLoS One 2011;6:e16245. doi:10.1371/journal.pone.0016245.

[222] Wakim LM, Gupta N, Mintern JD, Villadangos JA. Enhanced survival of lung tissue-resident memory $CD8^+$ T cells during infection with influenza virus due to selective expression of IFITM3. Nat Immunol 2013;14:238–45. doi:10.1038/ni.2525.

[223] Schenkel JM, Fraser KA, Vezys V, Masopust D. Sensing and alarm function of resident memory CD8+ T cells. Nat Immunol 2013;14:509–13. doi:10.1038/ni.2568.

[224] Schenkel JM, Fraser KA, Beura LK, Pauken KE, Vezys V, Masopust D. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. Science (80-) 2014;346:98–101. doi:10.1126/science.1254536.

[225] Wakim LM, Woodward-Davis A, Bevan MJ. Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence. Proc Natl Acad Sci 2010;107:17872–9. doi:10.1073/pnas.1010201107.

[226] Pallett LJ, Davies J, Colbeck EJ, Robertson F, Hansi N, Easom NJW, et al. IL-2(high) tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. J Exp Med 2017;214:1567–80. doi:10.1084/jem.20162115.

[227] Stelma F, de Niet A, Sinnige MJ, van Dort KA, van Gisbergen KPJM, Verheij J, et al. Human intrahepatic CD69 + CD8+ T cells have a tissue resident memory T cell phenotype with reduced cytolytic capacity. Sci Rep 2017;7:6172. doi:10.1038/s41598-017-06352-3.

[228] Pauls K, Schön M, Kubitza RC, Homey B, Wiesenborn A, Lehmann P, et al. Role of integrin alphaE(CD103)beta7 for tissue-specific epidermal localization of CD8+ T lymphocytes. J Invest Dermatol 2001;117:569–75. doi:10.1046/j.0022-202x.2001.01481.x.

[229] Mackay LK, Braun A, Macleod BL, Collins N, Tebartz C, Bedoui S, et al. Cutting Edge: CD69 Interference with Sphingosine-1-Phosphate Receptor Function Regulates Peripheral T Cell Retention. J Immunol 2015;194:2059–63. doi:10.4049/jimmunol.1402256.

[230] Ray SJ, Franki SN, Pierce RH, Dimitrova S, Koteliansky V, Sprague AG, et al. The collagen binding alpha1beta1 integrin VLA-1 regulates CD8 T cell-mediated immune protection against heterologous influenza infection. Immunity 2004;20:167–79.

[231] Zhang N, Bevan MJ. Transforming Growth Factor-β Signaling Controls the Formation and Maintenance of Gut-Resident Memory T Cells by Regulating Migration and Retention. Immunity 2013;39:687–96. doi:10.1016/j.immuni.2013.08.019.

[232] Shiow LR, Rosen DB, Brdičková N, Xu Y, An J, Lanier LL, et al. CD69 acts downstream of interferon-α/β to inhibit S1P1 and lymphocyte egress from lymphoid organs. Nature 2006;440:540–4. doi:10.1038/nature04606.

[233] Skon CN, Lee J-Y, Anderson KG, Masopust D, Hogquist KA, Jameson SC. Transcriptional downregulation of S1pr1 is required for the establishment of resident memory CD8+ T cells. Nat Immunol 2013;14:1285–93. doi:10.1038/ni.2745.

[234] Cepek KL, Shaw SK, Parker CM, Russell GJ, Morrow JS, Rimm DL, et al. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. Nature 1994;372:190–3. doi:10.1038/372190a0.

[235] Niessen CM. Tight Junctions/Adherens Junctions: Basic Structure and Function. J Invest Dermatol 2007;127:2525–32. doi:10.1038/sj.jid.5700865.

[236] Vandenberg P, Kern A, Ries A, Luckenbill-Edds L, Mann K, Kühn K. Characterization of a type IV collagen major cell binding site with affinity to the alpha 1 beta 1 and the alpha 2 beta 1 integrins. J Cell Biol 1991;113:1475–83.

[237] Eble JA, Golbik R, Mann K, Kühn K. The alpha 1 beta 1 integrin recognition site of the basement membrane collagen molecule [alpha 1(IV)]2 alpha 2(IV). EMBO J 1993;12:4795–802.

[238] Hemler ME, Sanchez-Madrid F, Flotte TJ, Krensky AM, Burakoff SJ, Bhan AK, et al. Glycoproteins of 210,000 and 130,000 m.w. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. J Immunol 1984;132:3011–8.

[239] Hemler ME, Jacobson JG, Brenner MB, Mann D, Strominger JL. VLA-1: a T cell surface antigen which defines a novel late stage of human T cell activation. Eur J Immunol 1985;15:502–8. doi:10.1002/eji.1830150515.

[240] Hemler ME, Glass D, Coblyn JS, Jacobson JG. Very late activation antigens on rheumatoid synovial fluid T lymphocytes. Association with stages of T cell activation. J Clin Invest 1986;78:696–702. doi:10.1172/JCI112629.

[241] Chapman TJ, Topham DJ. Identification of a Unique Population of Tissue-Memory CD4+ T Cells in the Airways after Influenza Infection That Is Dependent on the Integrin VLA-1. J Immunol 2010;184:3841–9. doi:10.4049/jimmunol.0902281.

[242] Piet B, de Bree GJ, Smids-Dierdorp BS, van der Loos CM, Remmerswaal EBM, von der Thüsen JH, et al. CD8+ T cells with an intraepithelial phenotype upregulate cytotoxic function upon influenza infection in human lung. J Clin Invest 2011;121:2254–63. doi:10.1172/JCI44675.

[243] Mackay LK, Wynne-Jones E, Freestone D, Pellicci DG, Mielke LA, Newman DM, et al. T-box Transcription Factors Combine with the Cytokines TGF-β and IL-15 to Control Tissue-Resident Memory T Cell Fate. Immunity 2015;43:1101–11. doi:10.1016/j.immuni.2015.11.008.

[244] Mackay LK, Minnich M, Kragten NAM, Liao Y, Nota B, Seillet C, et al. Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. Science (80-) 2016;352:459–63. doi:10.1126/science.aad2035.

[245] Jabri B, Abadie V. IL-15 functions as a danger signal to regulate tissue-resident T cells and tissue destruction. Nat Rev Immunol 2015;15:771–83. doi:10.1038/nri3919.

[246] Nestle FO, Di Meglio P, Qin J-Z, Nickoloff BJ. Skin immune sentinels in health and disease. Nat Rev Immunol 2009;9:679–91. doi:10.1038/nri2622.

[247] Foster CA, Yokozeki H, Rappersberger K, Koning F, Volc-Platzer B, Rieger A, et al. Human epidermal T cells predominantly belong to the lineage expressing alpha/beta T cell receptor. J Exp Med 1990;171:997–1013.

[248] Schuster C, Vaculik C, Prior M, Fiala C, Mildner M, Eppel W, et al. Phenotypic Characterization of Leukocytes in Prenatal Human Dermis. J Invest Dermatol 2012;132:2581–92. doi:10.1038/jid.2012.187.

[249] Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastenmuller W, et al. Compartmentalized Control of Skin Immunity by Resident Commensals. Science (80-) 2012;337:1115–9. doi:10.1126/science.1225152.

[250] Zhu J, Koelle DM, Cao J, Vazquez J, Huang ML, Hladik F, et al. Virus-specific CD8+ T

cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. J Exp Med 2007;204:595–603. doi:10.1084/jem.20061792.

[251] Zhu J, Peng T, Johnston C, Phasouk K, Kask AS, Klock A, et al. Immune surveillance by CD8αα+ skin-resident T cells in human herpes virus infection. Nature 2013;497:494–7. doi:10.1038/nature12110.

[252] Vukmanovic-Stejic M, Sandhu D, Seidel JA, Patel N, Sobande TO, Agius E, et al. The Characterization of Varicella Zoster Virus-Specific T Cells in Skin and Blood during Aging. J Invest Dermatol 2015;135:1752–62. doi:10.1038/jid.2015.63.

[253] Kupper TS, Fuhlbrigge RC, Kieffer JD, Armerding D. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. Nature 1997;389:978–81. doi:10.1038/40166.

[254] Clark RA, Chong B, Mirchandani N, Brinster NK, Yamanaka K-I, Dowgiert RK, et al. The vast majority of CLA+ T cells are resident in normal skin. J Immunol 2006;176:4431–9.

[255] Spetz AL, Strominger J, Groh-Spies V. T cell subsets in normal human epidermis. Am J Pathol 1996;149:665–74.

[256] Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. Nat Med 2015;21:688–97. doi:10.1038/nm.3883.

[257] Boehncke W-H, Schön MP. Psoriasis. Lancet (London, England) 2015;386:983–94. doi:10.1016/S0140-6736(14)61909-7.

[258] Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. Lancet 2015;386:74–84. doi:10.1016/S0140-6736(14)60763-7.

[259] Cheuk S, Wiken M, Blomqvist L, Nylen S, Talme T, Stahle M, et al. Epidermal Th22 and Tc17 Cells Form a Localized Disease Memory in Clinically Healed Psoriasis. J Immunol 2014;192:3111–20. doi:10.4049/jimmunol.1302313.

[260] van den Boorn JG, Konijnenberg D, Dellemijn TAM, van der Veen JPW, Bos JD, Melief CJM, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. J Invest Dermatol 2009;129:2220–32. doi:10.1038/jid.2009.32.

[261] Yang L, Wei Y, Sun Y, Shi W, Yang J, Zhu L, et al. Interferon-gamma Inhibits Melanogenesis and Induces Apoptosis in Melanocytes: A Pivotal Role of CD8+ Cytotoxic T Lymphocytes in Vitiligo. Acta Derm Venereol 2015;95:664–70. doi:10.2340/00015555-2080.

[262] Harris JE, Harris TH, Weninger W, John Wherry E, Hunter CA, Turka LA. A Mouse Model of Vitiligo with Focused Epidermal Depigmentation Requires IFN-γ for Autoreactive CD8+ T-Cell Accumulation in the Skin. J Invest Dermatol 2012;132:1869–76. doi:10.1038/jid.2011.463.

[263] Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su M-W, et al. CXCL10 Is Critical for the Progression and Maintenance of Depigmentation in a Mouse Model of Vitiligo. Sci Transl Med 2014;6:223ra23-223ra23. doi:10.1126/scitranslmed.3007811.

[264] Law RHP, Lukoyanova N, Voskoboinik I, Caradoc-Davies TT, Baran K, Dunstone MA, et al. The structural basis for membrane binding and pore formation by lymphocyte perforin. Nature 2010;468:447–51. doi:10.1038/nature09518.

[265] Voskoboinik I, Dunstone MA, Baran K, Whisstock JC, Trapani JA. Perforin: structure, function, and role in human immunopathology. Immunol Rev 2010;235:35–54. doi:10.1111/j.0105-2896.2010.00896.x.

[266] Brennan AJ, Chia J, Browne KA, Ciccone A, Ellis S, Lopez JA, et al. Protection from Endogenous Perforin: Glycans and the C Terminus Regulate Exocytic Trafficking in Cytotoxic Lymphocytes. Immunity 2011;34:879–92. doi:10.1016/j.immuni.2011.04.007.

[267] Uellner R, Zvelebil MJ, Hopkins J, Jones J, MacDougall LK, Morgan BP, et al. Perforin is activated by a proteolytic cleavage during biosynthesis which reveals a phospholipid-binding C2 domain. EMBO J 1997;16:7287–96. doi:10.1093/emboj/16.24.7287.

[268] Konjar S, Sutton VR, Hoves S, Repnik U, Yagita H, Reinheckel T, et al. Human and

mouse perforin are processed in part through cleavage by the lysosomal cysteine proteinase cathepsin L. Immunology 2010;131:257–67. doi:10.1111/j.1365-2567.2010.03299.x.

[269] Fraser SA, Karimi R, Michalak M, Hudig D. Perforin lytic activity is controlled by calreticulin. J Immunol 2000;164:4150–5.

[270] Metkar SS, Wang B, Aguilar-Santelises M, Raja SM, Uhlin-Hansen L, Podack E, et al. Cytotoxic cell granule-mediated apoptosis: perforin delivers granzyme B-serglycin complexes into target cells without plasma membrane pore formation. Immunity 2002;16:417–28.

[271] Cohnen A, Chiang SC, Stojanovic A, Schmidt H, Claus M, Saftig P, et al. Surface CD107a/LAMP-1 protects natural killer cells from degranulation-associated damage. Blood 2013;122:1411–8. doi:10.1182/blood-2012-07-441832.

[272] Aktas E, Kucuksezer UC, Bilgic S, Erten G, Deniz G. Relationship between CD107a expression and cytotoxic activity. Cell Immunol 2009;254:149–54. doi:10.1016/j.cellimm.2008.08.007.

[273] Alter G, Malenfant JM, Altfeld M. CD107a as a functional marker for the identification of natural killer cell activity. J Immunol Methods 2004;294:15–22. doi:10.1016/j.jim.2004.08.008.

[274] Smyth MJ, McGuire MJ, Thia KY. Expression of recombinant human granzyme B. A processing and activation role for dipeptidyl peptidase I. J Immunol 1995;154:6299–305.

[275] Pham CT, Ley TJ. Dipeptidyl peptidase I is required for the processing and activation of granzymes A and B in vivo. Proc Natl Acad Sci U S A 1999;96:8627–32.

[276] Pham CTN, Ivanovich JL, Raptis SZ, Zehnbauer B, Ley TJ. Papillon-Lefèvre syndrome: correlating the molecular, cellular, and clinical consequences of cathepsin C/dipeptidyl peptidase I deficiency in humans. J Immunol 2004;173:7277–81.

[277] Meade JL, de Wynter EA, Brett P, Sharif SM, Woods CG, Markham AF, et al. A family with Papillon-Lefevre syndrome reveals a requirement for cathepsin C in granzyme B activation and NK cell cytolytic activity. Blood 2006;107:3665–8. doi:10.1182/blood-2005-03-1140.

[278] D'Angelo ME, Bird PI, Peters C, Reinheckel T, Trapani JA, Sutton VR. Cathepsin H Is an Additional Convertase of Pro-granzyme B. J Biol Chem 2010;285:20514–9. doi:10.1074/jbc.M109.094573.

[279] Grossman WJ, Revell PA, Lu ZH, Johnson H, Bredemeyer AJ, Ley TJ. The orphan granzymes of humans and mice. Curr Opin Immunol 2003;15:544–52.

[280] Bengsch B, Ohtani T, Herati RS, Bovenschen N, Chang K-M, Wherry EJ. Deep immune profiling by mass cytometry links human T and NK cell differentiation and cytotoxic molecule expression patterns. J Immunol Methods 2017. doi:10.1016/j.jim.2017.03.009.

[281] Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol 2015;15:388–400. doi:10.1038/nri3839.

[282] Susanto O, Stewart SE, Voskoboinik I, Brasacchio D, Hagn M, Ellis S, et al. Mouse granzyme A induces a novel death with writhing morphology that is mechanistically distinct from granzyme B-induced apoptosis. Cell Death Differ 2013;20:1183–93. doi:10.1038/cdd.2013.59.

[283] Wensink AC, Hack CE, Bovenschen N. Granzymes Regulate Proinflammatory Cytokine Responses. J Immunol 2015;194:491–7. doi:10.4049/jimmunol.1401214.

[284] Metkar SS, Menaa C, Pardo J, Wang B, Wallich R, Freudenberg M, et al. Human and Mouse Granzyme A Induce a Proinflammatory Cytokine Response. Immunity 2008;29:720–33. doi:10.1016/j.immuni.2008.08.014.

[285] Stinchcombe JC, Bossi G, Booth S, Griffiths GM. The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity 2001;15:751–61.

[286] Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM. Centrosome polarization

delivers secretory granules to the immunological synapse. Nature 2006;443:462–5. doi:10.1038/nature05071.

[287] Kurowska M, Goudin N, Nehme NT, Court M, Garin J, Fischer A, et al. Terminal transport of lytic granules to the immune synapse is mediated by the kinesin-1/Slp3/Rab27a complex. Blood 2012;119:3879–89. doi:10.1182/blood-2011-09-382556.

[288] Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science 1999;286:1957–9.

[289] Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell 2003;115:461–73.

[290] zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter J-I, et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet 2005;14:827–34. doi:10.1093/hmg/ddi076.

[291] Côte M, Ménager MM, Burgess A, Mahlaoui N, Picard C, Schaffner C, et al. Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. J Clin Invest 2009;119:3765–73. doi:10.1172/JCI40732.

[292] Ménasché G, Pastural E, Feldmann J, Certain S, Ersoy F, Dupuis S, et al. Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. Nat Genet 2000;25:173–6. doi:10.1038/76024.

[293] Wood SM, Meeths M, Chiang SCC, Bechensteen AG, Boelens JJ, Heilmann C, et al. Different NK cell-activating receptors preferentially recruit Rab27a or Munc13-4 to perforin-containing granules for cytotoxicity. Blood 2009;114:4117–27. doi:10.1182/blood-2009-06-225359.

[294] Ménager MM, Ménasché G, Romao M, Knapnougel P, Ho C-H, Garfa M, et al. Secretory cytotoxic granule maturation and exocytosis require the effector protein hMunc13-4. Nat Immunol 2007;8:257–67. doi:10.1038/ni1431.

[295] Marshall MR, Pattu V, Halimani M, Maier-Peuschel M, Müller M-L, Becherer U, et al. VAMP8-dependent fusion of recycling endosomes with the plasma membrane facilitates T lymphocyte cytotoxicity. J Cell Biol 2015;210:135–51. doi:10.1083/jcb.201411093.

[296] Voss M, Bryceson YT. Natural killer cell biology illuminated by primary immunodeficiency syndromes in humans. Clin Immunol 2017;177:29–42. doi:10.1016/j.clim.2015.11.004.

[297] Meeths M, Chiang SCC, Wood SM, Entesarian M, Schlums H, Bang B, et al. Familial hemophagocytic lymphohistiocytosis type 3 (FHL3) caused by deep intronic mutation and inversion in UNC13D. Blood 2011;118:5783–93. doi:10.1182/blood-2011-07-369090.

[298] Entesarian M, Chiang SCC, Schlums H, Meeths M, Chan M.-Y, Mya S.-N, et al. Novel deep intronic and missense UNC13D mutations in familial haemophagocytic lymphohistiocytosis type 3. Br J Haematol 2013;162. doi:10.1111/bjh.12371.

[299] Seo JY, Song J-S, Lee K-O, Won H-H, Kim J-W, Kim S-H, et al. Founder effects in two predominant intronic mutations of UNC13D, c.118-308C>T and c.754-1G>C underlie the unusual predominance of type 3 familial hemophagocytic lymphohistiocytosis (FHL3) in Korea. Ann Hematol 2013;92:357–64. doi:10.1007/s00277-012-1628-6.

[300] Qian Y, Johnson JA, Connor JA, Valencia CA, Barasa N, Schubert J, et al. The 253-kb inversion and deep intronic mutations in UNC13D are present in North American patients with familial hemophagocytic lymphohistiocytosis 3. Pediatr Blood Cancer 2014;61:1034–40. doi:10.1002/pbc.24955.

[301] ALLFREY VG, FAULKNER R, MIRSKY AE. ACETYLATION AND METHYLATION OF

HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS. Proc Natl Acad Sci U S A 1964;51:786–94.

[302] Valouev A, Johnson SM, Boyd SD, Smith CL, Fire AZ, Sidow A. Determinants of nucleosome organization in primary human cells. Nature 2011;474:516–20. doi:10.1038/nature10002.

[303] Bell O, Tiwari VK, Thomä NH, Schübeler D. Determinants and dynamics of genome accessibility. Nat Rev Genet 2011;12:554–64. doi:10.1038/nrg3017.

[304] Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012;13:484–92. doi:10.1038/nrg3230.

[305] Jones PA, Liang G. Rethinking how DNA methylation patterns are maintained. Nat Rev Genet 2009;10:805–11. doi:10.1038/nrg2651.

[306] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999;99:247–57.

[307] Pastor WA, Aravind L, Rao A. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. Nat Rev Mol Cell Biol 2013;14:341–56. doi:10.1038/nrm3589.

[308] Li B, Carey M, Workman JL. The Role of Chromatin during Transcription. Cell 2007;128:707–19. doi:10.1016/j.cell.2007.01.015.

[309] Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NCT, et al. Active genes are tri-methylated at K4 of histone H3. Nature 2002;419:407–11. doi:10.1038/nature01080.

[310] Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res 2011;21:381–95. doi:10.1038/cr.2011.22.

[311] Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, et al. Combinatorial patterns of histone acetylations and methylations in the human genome. Nat Genet 2008;40:897–903. doi:10.1038/ng.154.

[312] Struhl K. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev 1998;12:599–606.

[313] Eberharter A, Becker PB. Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. EMBO Rep 2002;3:224–9. doi:10.1093/embo-reports/kvf053.

[314] Bird A, Nan X, Ng H-H, Johnson CA, Laherty CD, Turner BM, et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 1998;393:386–9. doi:10.1038/30764.

[315] Gray SM, Kaech SM, Staron MM. The interface between transcriptional and epigenetic control of effector and memory CD8(+) T-cell differentiation. Immunol Rev 2014;261:157–68. doi:10.1111/imr.12205.

[316] Dogra P, Ghoneim HE, Abdelsamed HA, Youngblood B. Generating long-lived CD8 + T-cell memory: Insights from epigenetic programs. Eur J Immunol 2016;46:1548–62. doi:10.1002/eji.201545550.

[317] Scharer CD, Barwick BG, Youngblood BA, Ahmed R, Boss JM. Global DNA Methylation Remodeling Accompanies CD8 T Cell Effector Function. J Immunol 2013;191:3419–29. doi:10.4049/jimmunol.1301395.

[318] Abdelsamed HA, Moustaki A, Fan Y, Dogra P, Ghoneim HE, Zebley CC, et al. Human memory CD8 T cell effector potential is epigenetically preserved during in vivo homeostasis. J Exp Med 2017;214:1593–606. doi:10.1084/jem.20161760.

[319] Rodriguez RM, Suarez-Alvarez B, Lavín JL, Mosén-Ansorena D, Baragaño Raneros A, Márquez-Kisinousky L, et al. Epigenetic Networks Regulate the Transcriptional Program in Memory and Terminally Differentiated CD8 $^{+}$ T Cells. J Immunol 2017;198:937–49. doi:10.4049/jimmunol.1601102.

[320] Fann M, Godlove JM, Catalfamo M, Wood WH, Chrest FJ, Chun N, et al. Histone

acetylation is associated with differential gene expression in the rapid and robust memory CD8+ T-cell response. Blood 2006;108:3363–70. doi:10.1182/blood-2006-02-005520.

[321] Araki Y, Fann M, Wersto R, Weng N-P. Histone acetylation facilitates rapid and robust memory CD8 T cell response through differential expression of effector molecules (eomesodermin and its targets: perforin and granzyme B). J Immunol 2008;180:8102–8.

[322] Northrop JK, Wells AD, Shen H. Cutting edge: chromatin remodeling as a molecular basis for the enhanced functionality of memory CD8 T cells. J Immunol 2008;181:865–8.

[323] Araki Y, Wang Z, Zang C, Wood WH, Schones D, Cui K, et al. Genome-wide Analysis of Histone Methylation Reveals Chromatin State-Based Regulation of Gene Transcription and Function of Memory CD8+ T Cells. Immunity 2009;30:912–25. doi:10.1016/j.immuni.2009.05.006.

[324] Russ BE, Olshanksy M, Smallwood HS, Li J, Denton AE, Prier JE, et al. Distinct epigenetic signatures delineate transcriptional programs during virus-specific CD8(+) T cell differentiation. Immunity 2014;41:853–65. doi:10.1016/j.immuni.2014.11.001.

[325] Denton AE, Russ BE, Doherty PC, Rao S, Turner SJ. Differentiation-dependent functional and epigenetic landscapes for cytokine genes in virus-specific CD8+ T cells. Proc Natl Acad Sci 2011;108:15306–11. doi:10.1073/pnas.1112520108.

[326] Zediak VP, Johnnidis JB, Wherry EJ, Berger SL. Cutting edge: persistently open chromatin at effector gene loci in resting memory CD8+ T cells independent of transcriptional status. J Immunol 2011;186:2705–9. doi:10.4049/jimmunol.1003741.

[327] Miller SA, Huang AC, Miazgowicz MM, Brassil MM, Weinmann AS. Coordinated but physically separable interaction with H3K27-demethylase and H3K4-methyltransferase activities are required for T-box protein-mediated activation of developmental gene expression. Genes Dev 2008;22:2980–93. doi:10.1101/gad.1689708.

[328] Vahedi G, Takahashi H, Nakayamada S, Sun H-W, Sartorelli V, Kanno Y, et al. STATs shape the active enhancer landscape of T cell populations. Cell 2012;151:981–93. doi:10.1016/j.cell.2012.09.044.

[329] Vahedi G, C. Poholek A, Hand TW, Laurence A, Kanno Y, O'Shea JJ, et al. Helper T-cell identity and evolution of differential transcriptomes and epigenomes. Immunol Rev 2013;252:24–40. doi:10.1111/imr.12037.

[330] Cichocki F, Miller JS, Anderson SK, Bryceson YT. Epigenetic regulation of NK cell differentiation and effector functions. Front Immunol 2013;4:55. doi:10.3389/fimmu.2013.00055.

[331] Anderson MK, Hernandez-Hoyos G, Diamond RA, Rothenberg E V. Precise developmental regulation of Ets family transcription factors during specification and commitment to the T cell lineage. Development 1999;126:3131–48.

[332] Youn BS, Kim KK, Kwon BS. A critical role of Sp1- and Ets-related transcription factors in maintaining CTL-specific expression of the mouse perforin gene. J Immunol 1996;157:3499–509.

[333] Lin JX, Bhat NK, John S, Queale WS, Leonard WJ. Characterization of the human interleukin-2 receptor beta-chain gene promoter: regulation of promoter activity by ets gene products. Mol Cell Biol 1993;13:6201–10.

[334] Lacorazza HD, Miyazaki Y, Di Cristofano A, Deblasio A, Hedvat C, Zhang J, et al. The ETS protein MEF plays a critical role in perforin gene expression and the development of natural killer and NK-T cells. Immunity 2002;17:437–49.

[335] Barton K, Muthusamy N, Fischer C, Ting CN, Walunas TL, Lanier LL, et al. The Ets-1 transcription factor is required for the development of natural killer cells in mice. Immunity 1998;9:555–63.

[336] Ramirez K, Chandler KJ, Spaulding C, Zandi S, Sigvardsson M, Graves BJ, et al. Gene Deregulation and Chronic Activation in Natural Killer Cells Deficient in the Transcription Factor ETS1. Immunity 2012;36:921–32. doi:10.1016/j.immuni.2012.04.006.

[337] Choi H-J, Geng Y, Cho H, Li S, Giri PK, Felio K, et al. Differential requirements for the Ets transcription factor Elf-1 in the development of NKT cells and NK cells. Blood 2011;117:1880–7. doi:10.1182/blood-2010-09-309468.

[338] Yang Y, Ochando JC, Bromberg JS, Ding Y. Identification of a distant T-bet enhancer responsive to IL-12/Stat4 and IFN /Stat1 signals. Blood 2007;110:2494–500. doi:10.1182/blood-2006-11-058271.

[339] Yamamoto K, Shibata F, Miyasaka N, Miura O. The human perforin gene is a direct target of STAT4 activated by IL-12 in NK cells. Biochem Biophys Res Commun 2002;297:1245–52.

[340] Zhang F, Boothby M. T helper type 1-specific Brg1 recruitment and remodeling of nucleosomes positioned at the IFN-gamma promoter are Stat4 dependent. J Exp Med 2006;203:1493–505. doi:10.1084/jem.20060066.

[341] Letimier FA, Passini N, Gasparian S, Bianchi E, Rogge L. Chromatin remodeling by the SWI/SNF-like BAF complex and STAT4 activation synergistically induce IL-12Rbeta2 expression during human Th1 cell differentiation. EMBO J 2007;26:1292–302. doi:10.1038/sj.emboj.7601586.

[342] Simon JM, Giresi PG, Davis IJ, Lieb JD. Using formaldehyde-assisted isolation of regulatory elements (FAIRE) to isolate active regulatory DNA. Nat Protoc 2012;7:256–67. doi:10.1038/nprot.2011.444.

[343] Kaplan MH, Sun Y-L, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature 1996;382:174–7. doi:10.1038/382174a0.

[344] Wu C, Ferrante J, Gately MK, Magram J. Characterization of IL-12 receptor beta1 chain (IL-12Rbeta1)-deficient mice: IL-12Rbeta1 is an essential component of the functional mouse IL-12 receptor. J Immunol 1997;159:1658–65.

[345] Presky DH, Minetti LJ, Gillessen S, Wilkinson VL, Wu CY, Gubler U, et al. Analysis of the multiple interactions between IL-12 and the high affinity IL-12 receptor complex. J Immunol 1998;160:2174–9. doi:10.4049/jimmunol.165.11.6221.

[346] Bacon CM, Petricoin EF, Ortaldo JR, Rees RC, Larner AC, Johnston JA, et al. Interleukin 12 induces tyrosine phosphorylation and activation of STAT4 in human lymphocytes. Proc Natl Acad Sci U S A 1995;92:7307–11.

[347] Gil MP, Ploquin MJY, Watford WT, Lee S-H, Kim K, Wang X, et al. Regulating type 1 IFN effects in CD8 T cells during viral infections: changing STAT4 and STAT1 expression for function. Blood 2012;120:3718–28. doi:10.1182/blood-2012-05-428672.

[348] Chueh F-Y, Yu C-L. Engagement of T-cell antigen receptor and CD4/CD8 co-receptors induces prolonged STAT activation through autocrine/paracrine stimulation in human primary T cells. Biochem Biophys Res Commun 2012;426:242–6. doi:10.1016/j.bbrc.2012.08.074.

[349] Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, et al. A human memory T cell subset with stem cell–like properties. Nat Med 2011;17:1290–7. doi:10.1038/nm.2446.

[350] Jiang D-K, Sun J, Cao G, Liu Y, Lin D, Gao Y-Z, et al. Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus–related hepatocellular carcinoma. Nat Genet 2012;45:72–5. doi:10.1038/ng.2483.

[351] Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. *STAT4* and the Risk of Rheumatoid Arthritis and Systemic Lupus Erythematosus. N Engl J Med 2007;357:977–86. doi:10.1056/NEJMoa073003.

[352] Korman BD, Alba MI, Le JM, Alevizos I, Smith JA, Nikolov NP, et al. Variant form of STAT4 is associated with primary Sjögren's syndrome. Genes Immun 2008;9:267–70. doi:10.1038/gene.2008.1.

[353] Lee H-S, Park H, Yang S, Kim D, Park Y. STAT4 Polymorphism Is Associated with Early-Onset Type 1 Diabetes, but Not with Late-Onset Type 1 Diabetes. Ann N Y Acad Sci 2008;1150:93–8. doi:10.1196/annals.1447.013.

[354] Fauriat C, Long EO, Ljunggren H-G, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood 2010;115:2167–76. doi:10.1182/blood-2009-08-238469.

[355] Colucci F, Turner M, Schweighoffer E, Guy-Grand D, Di Bartolo V, Salcedo M, et al. Redundant role of the Syk protein tyrosine kinase in mouse NK cell differentiation. J Immunol 1999;163:1769–74.

[356] Veillette A. The SAP Family: A New Class of Adaptor-Like Molecules That Regulates Immune Cell Functions. Sci Signal 2002;2002:pe8-pe8. doi:10.1126/stke.2002.120.pe8.

[357] Kersh EN, Fitzpatrick DR, Murali-Krishna K, Shires J, Speck SH, Boss JM, et al. Rapid demethylation of the IFN-gamma gene occurs in memory but not naive CD8 T cells. J Immunol 2006;176:4083–93.

[358] Mathew R, Seiler MP, Scanlon ST, Mao A, Constantinides MG, Bertozzi-Villa C, et al. BTB-ZF factors recruit the E3 ligase cullin 3 to regulate lymphoid effector programs. Nature 2012;491:618–21. doi:10.1038/nature11548.

[359] Lee S-U, Maeda T. POK/ZBTB proteins: an emerging family of proteins that regulate lymphoid development and function. Immunol Rev 2012;247:107–19. doi:10.1111/j.1600-065X.2012.01116.x.

[360] Puszyk W, Down T, Grimwade D, Chomienne C, Oakey RJ, Solomon E, et al. The epigenetic regulator PLZF represses L1 retrotransposition in germ and progenitor cells. EMBO J 2013;32:1941–52. doi:10.1038/emboj.2013.118.

[361] Kovalovsky D, Uche OU, Eladad S, Hobbs RM, Yi W, Alonzo E, et al. The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions. Nat Immunol 2008;9:1055–64. doi:10.1038/ni.1641.

[362] Savage AK, Constantinides MG, Han J, Picard D, Martin E, Li B, et al. The transcription factor PLZF directs the effector program of the NKT cell lineage. Immunity 2008;29:391–403. doi:10.1016/j.immuni.2008.07.011.

[363] Kreslavsky T, Savage AK, Hobbs R, Gounari F, Bronson R, Pereira P, et al. TCR-inducible PLZF transcription factor required for innate phenotype of a subset of T cells with restricted TCR diversity. Proc Natl Acad Sci 2009;106:12453–8. doi:10.1073/pnas.0903895106.

[364] Gleimer M, von Boehmer H, Kreslavsky T. PLZF Controls the Expression of a Limited Number of Genes Essential for NKT Cell Function. Front Immunol 2012;3:374. doi:10.3389/fimmu.2012.00374.

[365] Sáez-Borderías A, Romo N, Magri G, Gumá M, Angulo A, López-Botet M. IL-12-dependent inducible expression of the CD94/NKG2A inhibitory receptor regulates CD94/NKG2C+ NK cell function. J Immunol 2009;182:829–36.

[366] Shin HM, Kapoor VN, Kim G, Li P, Kim H-R, Suresh M, et al. Transient expression of ZBTB32 in anti-viral CD8+ T cells limits the magnitude of the effector response and the generation of memory. PLOS Pathog 2017;13:e1006544. doi:10.1371/journal.ppat.1006544.

[367] Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Münz C. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. J Exp Med 2002;195:343–51.

[368] Waggoner SN, Cornberg M, Selin LK, Welsh RM. Natural killer cells act as rheostats modulating antiviral T cells. Nature 2011;481:394–8. doi:10.1038/nature10624.

[369] Crouse J, Bedenikovic G, Wiesel M, Ibberson M, Xenarios I, Von Laer D, et al. Type I interferons protect T cells against NK cell attack mediated by the activating receptor NCR1. Immunity 2014;40:961–73. doi:10.1016/j.immuni.2014.05.003.

[370] Xu HC, Grusdat M, Pandyra AA, Polz R, Huang J, Sharma P, et al. Type I Interferon Protects Antiviral CD8+ T Cells from NK Cell Cytotoxicity. Immunity 2014;40:949–60. doi:10.1016/j.immuni.2014.05.004.

[371] Rydyznski C, Daniels KA, Karmele EP, Brooks TR, Mahl SE, Moran MT, et al.

Generation of cellular immune memory and B-cell immunity is impaired by natural killer cells. Nat Commun 2015;6:6375. doi:10.1038/ncomms7375.

[372] Kazmin D, Nakaya HI, Lee EK, Johnson MJ, van der Most R, van den Berg RA, et al. Systems analysis of protective immune responses to RTS,S malaria vaccination in humans. Proc Natl Acad Sci U S A 2017;114:2425–30. doi:10.1073/pnas.1621489114.

[373] Stemberger C, Huster KM, Koffler M, Anderl F, Schiemann M, Wagner H, et al. A Single Naive CD8+ T Cell Precursor Can Develop into Diverse Effector and Memory Subsets. Immunity 2007;27:985–97. doi:10.1016/j.immuni.2007.10.012.

[374] Buchholz VR, Flossdorf M, Hensel I, Kretschmer L, Weissbrich B, Graf P, et al. Disparate Individual Fates Compose Robust CD8+ T Cell Immunity. Science (80-) 2013;340:630–5. doi:10.1126/science.1235454.

[375] Gerlach C, Rohr JC, Perie L, van Rooij N, van Heijst JWJ, Velds A, et al. Heterogeneous Differentiation Patterns of Individual CD8+ T Cells. Science (80-) 2013;340:635–9. doi:10.1126/science.1235487.

[376] Arsenio J, Kakaradov B, Metz PJ, Kim SH, Yeo GW, Chang JT. Early specification of CD8+ T lymphocyte fates during adaptive immunity revealed by single-cell gene-expression analyses. Nat Immunol 2014;15:365–72. doi:10.1038/ni.2842.

[377] Freund J, May RM, Yang E, Li H, McCullen M, Zhang B, et al. Activating Receptor Signals Drive Receptor Diversity in Developing Natural Killer Cells. PLOS Biol 2016;14:e1002526. doi:10.1371/journal.pbio.1002526.

[378] Liu LL, Landskron J, Ask EH, Enqvist M, Sohlberg E, Traherne JA, et al. Critical Role of CD2 Co-stimulation in Adaptive Natural Killer Cell Responses Revealed in NKG2C-Deficient Humans. Cell Rep 2016;15:1088–99. doi:10.1016/j.celrep.2016.04.005.

[379] Tesi B, Schlums H, Cichocki F, Bryceson YT. Epigenetic Regulation of Adaptive NK Cell Diversification. Trends Immunol 2016;37. doi:10.1016/j.it.2016.04.006.

[380] Narni-Mancinelli E, Jaeger BN, Bernat C, Fenis A, Kung S, De Gassart A, et al. Tuning of Natural Killer Cell Reactivity by NKp46 and Helios Calibrates T Cell Responses. Science (80-) 2012;335:344–8. doi:10.1126/science.1215621.

[381] Deng W, Gowen BG, Zhang L, Wang L, Lau S, Iannello A, et al. A shed NKG2D ligand that promotes natural killer cell activation and tumor rejection. Science (80-) 2015;348:136–9. doi:10.1126/science.1258867.

[382] Surh CD, Sprent J. Homeostasis of Naive and Memory T Cells. Immunity 2008;29:848–62. doi:10.1016/j.immuni.2008.11.002.

[383] Yoshida T, Mei H, Dörner T, Hiepe F, Radbruch A, Fillatreau S, et al. Memory B and memory plasma cells. Immunol Rev 2010;237:117–39. doi:10.1111/j.1600-065X.2010.00938.x.

[384] Richards SJ, Norfolk DR, Swirsky DM, Hillmen P. Lymphocyte subset analysis and glycosylphosphatidylinositol phenotype in patients with paroxysmal nocturnal hemoglobinuria. Blood 1998;92:1799–806.

[385] El-Sherbiny YM, Doody GM, Kelly RJ, Hill A, Hillmen P, Cook GP. Natural killer (NK) cell function in paroxysmal nocturnal hemoglobinuria: a deficiency of NK cells, but not an NK cell deficiency. Blood 2015;125:1351–2. doi:10.1182/blood-2014-07-591255.

[386] Pu JJ, Brodsky RA. Paroxysmal Nocturnal Hemoglobinuria from Bench to Bedside. Clin Transl Sci 2011;4:219–24. doi:10.1111/j.1752-8062.2011.00262.x.

[387] El-Sherbiny YM, Kelly RJ, Hill A, Doody GM, Hillmen P, Cook GP. Altered natural killer cell subset homeostasis and defective chemotactic responses in paroxysmal nocturnal hemoglobinuria. Blood 2013;122:1887–90. doi:10.1182/blood-2013-06-507574.

[388] El-Sherbiny YM, Kelly RJ, Hill A, Doody GM, Hillmen P, Cook GP. Altered natural killer cell subset homeostasis and defective chemotactic responses in paroxysmal nocturnal hemoglobinuria. Blood 2013;122:1887–90. doi:10.1182/blood-2013-06-507574.

[389] Nuñez G, Hockenbery D, McDonnell TJ, Sorensen CM, Korsmeyer SJ. Bcl-2 maintains

B cell memory. Nature 1991;353:71–3. doi:10.1038/353071a0.

[390] Akbar AN, Salmon M, Savill J, Janossy G. A possible role for bcl-2 in regulating T-cell memory--a “balancing act” between cell death and survival. Immunol Today 1993;14:526–32. doi:10.1016/0167-5699(93)90181-J.

[391] Grayson JM, Zajac AJ, Altman JD, Ahmed R. Cutting edge: increased expression of Bcl-2 in antigen-specific memory CD8+ T cells. J Immunol 2000;164:3950–4.

[392] Wu T-S, Lee J-M, Lai Y-G, Hsu J-C, Tsai C-Y, Lee Y-H, et al. Reduced expression of Bcl-2 in CD8+ T cells deficient in the IL-15 receptor alpha-chain. J Immunol 2002;168:705–12.

[393] Berard M, Brandt K, Bulfone-Paus S, Tough DF. IL-15 promotes the survival of naive and memory phenotype CD8+ T cells. J Immunol 2003;170:5018–26.

[394] Huntington ND, Puthalakath H, Gunn P, Naik E, Michalak EM, Smyth MJ, et al. Interleukin 15–mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. Nat Immunol 2007;8:856–63. doi:10.1038/ni1487.

[395] Gérart S, Sibéril S, Martin E, Lenoir C, Aguilar C, Picard C, et al. Human iNKT and MAIT cells exhibit a PLZF-dependent proapoptotic propensity that is counterbalanced by XIAP. Blood 2013;121:614–23. doi:10.1182/blood-2012-09-456095.

[396] O’Sullivan TE, Johnson LR, Kang HH, Sun JC. BNIP3- and BNIP3L-Mediated Mitophagy Promotes the Generation of Natural Killer Cell Memory. Immunity 2015;43:331–42. doi:10.1016/j.immuni.2015.07.012.

[397] Mueller SN, Mackay LK. Tissue-resident memory T cells: local specialists in immune defence. Nat Rev Immunol 2015;16:79–89. doi:10.1038/nri.2015.3.

[398] Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat Immunol 2009;10:524–30. doi:10.1038/ni.1718.

[399] Fehniger TA, Cai SF, Cao X, Bredemeyer AJ, Presti RM, French AR, et al. Acquisition of Murine NK Cell Cytotoxicity Requires the Translation of a Pre-existing Pool of Granzyme B and Perforin mRNAs. Immunity 2007;26:798–811. doi:10.1016/j.immuni.2007.04.010.

[400] Banchereau J, Thompson-Snipes L, Zurawski S, Blanck J-P, Cao Y, Clayton S, et al. The differential production of cytokines by human Langerhans cells and dermal CD14+ DCs controls CTL priming. Blood 2012;119:5742–9. doi:10.1182/blood-2011-08-371245.

[401] Mohamadzadeh M, Takashima A, Dougherty I, Knop J, Bergstresser PR, Cruz PD. Ultraviolet B radiation up-regulates the expression of IL-15 in human skin. J Immunol 1995;155:4492–6.

[402] Akgül B, Cooke JC, Storey A. HPV-associated skin disease. J Pathol 2006;208:165–75. doi:10.1002/path.1893.

[403] Chisholm C, Lopez L. Cutaneous Infections Caused by Herpesviridae: A Review. Arch Pathol Lab Med 2011;135:1357–62. doi:10.5858/arpa.2010-0156-RS.

[404] Giustizieri ML, Mascia F, Frezzolini A, De Pità O, Chinni LM, Giannetti A, et al. Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. J Allergy Clin Immunol 2001;107:871–7. doi:10.1067/mai.2001.114707.

[405] Adachi T, Kobayashi T, Sugihara E, Yamada T, Ikuta K, Pittaluga S, et al. Hair follicle-derived IL-7 and IL-15 mediate skin-resident memory T cell homeostasis and lymphoma. Nat Med 2015;21:1272–9. doi:10.1038/nm.3962.

[406] Teunissen MB, Koomen CW, de Waal Malefyt R, Wierenga EA, Bos JD. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. J Invest Dermatol 1998;111:645–9. doi:10.1046/j.1523-1747.1998.00347.x.

[407] Blauvelt A, Asada H, Klaus-Kovtun V, Altman DJ, Lucey DR, Katz SI. Interleukin-15 mRNA is expressed by human keratinocytes Langerhans cells, and blood-derived dendritic cells and is downregulated by ultraviolet B radiation. J Invest Dermatol 1996;106:1047–52.

[408] Craiglow BG, King BA. Tofacitinib Citrate for the Treatment of Vitiligo. JAMA Dermatology 2015;151:1110. doi:10.1001/jamadermatol.2015.1520.

[409] Villadsen LS, Schuurman J, Beurskens F, Dam TN, Dagnæs-Hansen F, Skov L, et al. Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model. J Clin Invest 2003;112:1571–80. doi:10.1172/JCI18986.

[410] Xing L, Dai Z, Jabbari A, Cerise JE, Higgins CA, Gong W, et al. Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition. Nat Med 2014;20:1043–9. doi:10.1038/nm.3645.

[411] Conlon KC, Lugli E, Welles HC, Rosenberg SA, Fojo AT, Morris JC, et al. Redistribution, Hyperproliferation, Activation of Natural Killer Cells and CD8 T Cells, and Cytokine Production During First-in-Human Clinical Trial of Recombinant Human Interleukin-15 in Patients With Cancer. J Clin Oncol 2015;33:74–82. doi:10.1200/JCO.2014.57.3329.

[412] Robinson TO, Schluns KS. The potential and promise of IL-15 in immuno-oncogenic therapies. Immunol Lett 2017;190:159–68. doi:10.1016/j.imlet.2017.08.010.

[413] Hüe S, Mention J-J, Monteiro RC, Zhang S, Cellier C, Schmitz J, et al. A Direct Role for NKG2D/MICA Interaction in Villous Atrophy during Celiac Disease. Immunity 2004;21:367–77. doi:10.1016/j.immuni.2004.06.018.

[414] Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity 2004;21:357–66. doi:10.1016/j.immuni.2004.06.020.

[415] Zhang J, Basher F, Wu JD. NKG2D Ligands in Tumor Immunity: Two Sides of a Coin. Front Immunol 2015;6:97. doi:10.3389/fimmu.2015.00097.

[416] Paschen A, Baingo J, Schadendorf D. Expression of stress ligands of the immunoreceptor NKG2D in melanoma: Regulation and clinical significance. Eur J Cell Biol 2014;93:49–54. doi:10.1016/j.ejcb.2014.01.009.

[417] Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, et al. Regulation of Cutaneous Malignancy by gamma delta T Cells. Science (80-) 2001;294:605–9. doi:10.1126/science.1063916.

[418] Çuburu N, Graham BS, Buck CB, Kines RC, Pang Y-YS, Day PM, et al. Intravaginal immunization with HPV vectors induces tissue-resident CD8+ T cell responses. J Clin Invest 2012;122:4606–20. doi:10.1172/JCI63287.

[419] Sandoval F, Terme M, Nizard M, Badoual C, Bureau M-F, Freyburger L, et al. Mucosal Imprinting of Vaccine-Induced CD8+ T Cells Is Crucial to Inhibit the Growth of Mucosal Tumors. Sci Transl Med 2013;5:172ra20-172ra20. doi:10.1126/scitranslmed.3004888.